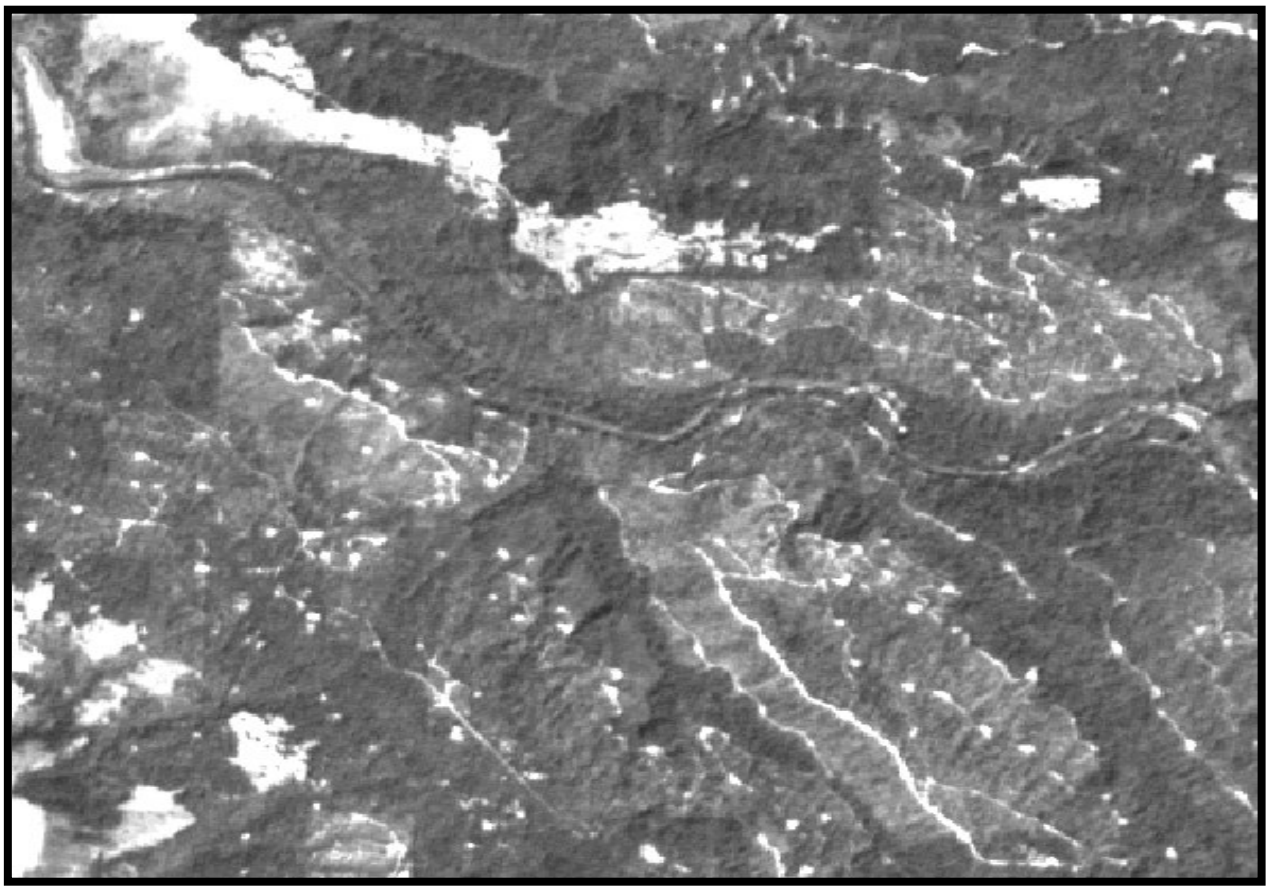
NORTH COAST RIVER LOADING STUDY
ROAD CROSSING ON SMALL STREAMS
VOLUME I. STATUS OF SALMONIDS IN THE WATERSHED



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EXECUTIVE SUMMARY

Commissioned by the California Department of Transportation, this study addressed the following primary objectives: 1) determine the absolute and relative contribution of Caltrans to the stressor loads within the watershed, and 2) determine if the timing, magnitude, and duration of Caltran's inputs were proportionately or disproportionately responsible for the decline of salmonids and the continuing degradation of salmonid habitat within the watershed. The research performed during the last three years may appear to be indirectly related to the measurement of stressors in the watershed. However, when regulations are imposed, it is necessary to demonstrate that those regulations (i.e., loads) adequately protect the resources at risk. Consequently, our research has focused on understanding the background levels of stressors, how those stressors have changed during the period in which salmonid declines have been observed, the mechanism by which those stressors impact salmonids, and finally, the origin of the stressors.

The Navarro watershed studies focused on five streams and their watersheds: Rancheria Creek, Anderson Creek, Indian Creek, North Fork, and Flynn Creek. All five sub-watersheds drain directly to the narrow floodplain of the Navarro River that runs next to Highway 128. Within each of the study drainages research activities were generally confined to each of three stream segments located in the upper, middle and lower portion of each watershed.

Two species of anadromous fish are currently found in the Navarro River watershed, coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*Oncorhynchus mykiss*). Other common fish species in the watershed include: California roach (*Lavinia symmetricus navarroensis*), three-spined stickleback (*Gasterosteus aculeatus aculeatus*), Sacramento sucker (*Catostomus occidentalis*), coast range sculpin (*Cottus aleuticus*), prickly sculpin (*Cottus asper*), pacific brook lamprey (*Lampetra pacifica*), and pacific lamprey (*Lampetra tridentata*). The California roach is the most common species of fish throughout the watershed.

The ratio Sr:Ca at the edges and in the cores of the otoliths of steelhead were measured to determine if the *O. mykiss* in the watershed were anadromous or resident. The distribution of values of the difference in Sr:Ca between the edge and the core indicate that the *O. mykiss* in the Navarro system are anadromous. There is a broad range of values (difference in Sr:Ca ratio between edge and core) that could be the result of three factors: 1) prolonged residence of females in the estuary prior to a spawning run upstream; 2) differential growth and metabolic demand at the early juvenile stage; and 3) increased stress on juvenile fish. Preliminary analyses indicate that the broad range of difference values most probably result from prolonged residence in the estuary by females prior to movement upstream.

Spawning occurred in all three years surveyed. Coho were primarily restricted to the North Fork and Flynn Creeks, although spawning coho were also seen in Indian Creek. Steelhead spawning occurred in all subwatersheds and there were as many spawning

steelhead in Anderson and Indian Creeks as in the North Fork. The winter of 2000-01 was the best year for both species, and the winter of 2001-02 was the worst year. As was the case for spawning, snorkel surveys indicated that there were more juvenile fish present in the watershed during the summer of 2001 compared to 2000. The most productive subwatershed was Rancheria Creek followed by Indian Creek, Anderson Creek, North Fork, and Flynn Creeks.

The earliest emergence date in 2000 was March 3, and the latest emergence date was June 16. The bulk of the fish emerged during April. There appeared to be no pattern in emergence with respect to sites within watersheds, in general fish emerged at each site across the entire period of emergence. Otoliths were used to obtain growth rates for fish collected during both summers. Currently, otoliths from 343 fish from the summer of 2000 have been photographed, and the daily growth rings delineated and measured. Fork lengths for each fish for each day of its life were calculated using the technique of Morita and Matsuishi (2001), and the growth rates were calculated for each fish.

When examined across all subwatersheds, growth rates were generally normally distributed with a mean daily growth rate of .51 mm/day (± .22mm/day StDev). Mean growth rate was negatively correlated with fish age ($r = -.40$, $df = 342$, $p = 0.000$) indicating that the older the fish, the slower the growth. When examined by individual subwatershed, there are substantial differences in the relationships between growth rate and age and date of emergence. The correlation of growth rate with age is always negative in all subwatersheds, although not always significantly. The correlation of

growth rate with emergence date is positive, negative, or nonexistent indicating that site-specific factors are important in setting growth rates.

Genetic analysis of microsatellite loci was performed. Average observed heterozygosities for pooled (range = 0.783-0.888) and discrete (0.745- 0.888) samples calculated across loci were similar to those seen in other population genetic studies utilizing microsatellites in steelhead trout from northern British Columbia, southern British Columbia, Washington, the Columbia River, and the Middle Fork Eel River in California. AMOVA results and pairwise population F_{st} values for the pooled samples indicated significant differences in genetic variation among the six Navarro River tributaries, suggesting limited contemporary gene flow among tributaries in the Navarro watershed. AMOVA results for the discrete samples, however, also indicated significant differences at the within-creek level; this accounted for an amount of the overall variance equal to that explained by the among-creek level. In addition, pairwise population F_{st} values indicated significant differences between some within-creek sites, as well as non-significance between some sites from different creeks. These small sizes may have generated sample allele frequency distributions that did not accurately reflect those of the real populations, and resulted in apparent within-creek differences in genetic variation. In any case, failure to account for within-creek heterogeneity would have led to an inflated value for the percentage of variance ascribed to among–creek variation (2.92 vs. 1.89).

Genetic distances between Navarro river tributaries were comparable to those reported in the literature for other steelhead populations. The relationships among Navarro River tributaries based on the analysis of pooled samples were quite robust and in complete accord with geographic distances among tributaries. These relationships broke down to some extent based on the discrete sample analysis, however, indicating that larger within-creek sample sizes and multiple-year samples may be required to confirm the results presented here.

Spatially unique stable isotope signatures, and correlation between invertebrate and steelhead δ-^{13}C values indicate that in Navarro Watershed and estuary, local environmental signals systematically determine consumer isotopic signatures. Steelhead δ-^{13}C values are related to the volume of habitats and the drainage area of the watershed they occupy. Boundaries of top predator ranges appear to be limited during late summer. Movement of invertebrates and parr may be limited at this time by low flow and shallow riffles in streams. Food chain length did not differ with average parr size, but food chain lengths to 1+ fish were an average of 1.04 delta units (or 0.3 trophic levels) higher than chain lengths to 0+ age fish. In the North Fork, average δ^{15}N values of smolts differed from parr by a full trophic level (3.8 δ^{15}N units). Thus, larger fish appear to consume organisms from higher trophic levels. Results of logistic regression from diet analysis suggest fish may be an important source of higher trophic status among large parr.

Sediment analysis was performed to determine historic sediment deposition rates over the last several thousand years, with an emphasis on the sediment deposition over the last

250 years. In addition, we determined the number of sites within the North Fork subwatershed that could deliver sediment to the river network. History of land use in the Navarro watershed is very recent. Although many forms of land use occur within the basin, logging activities have had the greatest impact in terms of magnitude of change. After settlement in the 1850's, *Sequoia* stands were gradually logged through the turn of the century (Palmer 1967, Holmes 1996), and an aerial photograph documents that much of the North Fork basin was deforested by a wildfire and logging in 1936. A third cut of the North Fork basin began in the 1990's, the extent of which is undetermined (Mendocino Redwood Company 2000).

A number of studies provide data showing the increased likelihood for landsliding after logging (*e.g.*, Sidle et al. 1985) and support the ability of floodplains to record anthropogenic disturbance as increased overbank deposition (Knox 1987, Marron 1992). However, based on long-term, net-averaged sedimentation rates, it appears that floodplains in the Navarro basin have not experienced increased sedimentation caused by disturbances to the landscape, at least over the time scales investigated by this study. The overbank deposition rates observed in this study are part of a general declining trend in sedimentation during the Holocene as a result of decreased precipitation and an exhaustion of sediment supplies.

A total of 1,065 erosional features were identified in the North Fork basin related to land uses such as logging and associated road networks, while a total of 38 features were identified in road cuts along Highway 128 from Dimmick State Park to where

Highway128 crosses the North Fork in the North Fork basin. The delivery ratio, an estimate of the connectivity between the sediment eroded from these features and the channel network, of 66% is estimated for slides the North Fork basin. Because of the proximity of Highway 128 to the main channel and floodplain of the North Fork, the delivery ratio from these sources was estimated at 100%. Results of this study suggest that the volume of sediment produced by erosional sources along the four mile length of Highway 128 within the North Fork basin account for a small fraction (0.3 %) of the total volume of sediment produced by erosional sources related to other land uses or natural causes in the remainder of the North Fork basin.

Our analyses support the hypothesis that logging practices have produced sediment pulses that travel rapidly through the Flynn Creek basin and imply that the system rapidly responds to and recovers from disturbance given enough time between logging periods. This is further evidenced by our high-resolution sedimentation data in that after 1850 and 1930, overbank deposition increased by as much as 7 and 13 times, respectively, before declining to rates slightly lower than antecedent conditions. Rapid return to antecedent conditions is likely a result of exhaustion of upstream sediment supply and/or hillslope stabilization by forest regrowth. Even though sediment loading recovered to near-normal levels after logging, the forest composition changed significantly and shows no trend towards return to antecedent conditions.

In our evaluation of the impacts of temperature on salmonids, we approached the problem in two ways. First, we inserted temperature probes in numerous reaches throughout the

watershed including all of the primary stations at which data were collected on fish abundance. We used these data to examine the relationship between various measures of temperature (e.g., maximum, daily range, average daily, average weekly, number of hours with temperatures above an 18°C threshold) and abundance of juvenile salmonids. We then extensively instrumented four pools in 2001, and three pools in 2002. Results indicate that changing the air temperature by 1°C only results in a change in water temperature of about 0.05°C, a very slight change. Consequently, to reduce the temperature of the water approximately 1°C, a decrease in air temperature of 20°C would be required. Our results indicate that once heated, it is very difficult to reduce the temperature of the water in the pools. Hyporheic water, which is traditionally thought to be cooler than surface water was actually warmer in our pools. Analyses are being completed which will indicate the source of heat to the stream.

Studies were undertaken to determine if biotic stressors have a significant impact on steelhead. Both competition with California roach and predation by birds was investigated. Interspecific competition with adult California roach had no measurable effect on 0+ steelhead trout growth. However, intraspecific competition had a large effect on steelhead trout growth; steelhead trout gained the most weight in low-density experiments. However, since California roach can tolerate water temperatures that induce physiological stress in steelhead trout (Moyle 2002, Werner et al. in prep.), they have the potential to gain a competitive advantage through exploitation competition at elevated water temperatures. Continuing anthropogenic modification of the stream system and surrounding watershed (e.g. surface and groundwater pumping, forest

removal, suburbanization) is creating more stream habitats that are shallower, warmer, less shaded, and thus more favorable for California roach and more stressful for steelhead trout. The increasing preponderance of exposed, warm water environments in the Navarro system has the potential to negatively affect steelhead trout directly through increased physiological stress and indirectly by giving California roach a competitive advantage.

It appears extremely unlikely that avian predators are having a significant impact on the steelhead or coho in this watershed. Most bird species feed on the most abundant prey species, and show no selectivity for given species. Older steelhead and coho occur in such low densities that birds are not likely to be cueing in on them as prey, but rather consuming mostly the more abundant 0+ steelhead, California roach, and three-spined sticklebacks. Assuming birds are consuming prey in relation to their densities, birds account for at most 8% of the decrease in this size class from June to July. In a worst-case scenario where birds are foraging exclusively on 0+ steelhead, they still only account for between 8-21% of decreased numbers between June and July. Although we do not have information on the diets of birds during out study, previous diet studies indicate that this later scenario is extremely unlikely, and that the former is a more realistic assessment of potential impacts. If anything, predation rates are likely to be lower than these estimates. The one place where predation may be an important source of mortality is at the estuary.

The presence of high water temperatures at locations throughout the watershed do not necessarily indicate that the fish are exposed to warmer water. We undertook an investigation to determine if fish were being exposed to higher water temperatures during the period of early development (fluctuating asymmetry) and during the juvenile portion of their life. Once it was established that the fish were being exposed to high water temperatures, we wanted to determine the mechanism(s) by which the stressors were impacting the salmonid populations. We undertook a study of the sublethal effects of temperature and zinc, the only inorganic contaminant that was found at elevated levels in the watershed. The temperature thresholds established in this study concur with what little is known about the sublethal consequences of exposure to elevated temperatures in *O. mykiss*. Based on the existing information on thermal tolerance of steelhead trout, the relative lack of toxicological stressors in the Navarro watershed and the pattern of increased hsp72 levels in fish at warmer sites, we conclude that the juvenile fish caught at Lower, Middle and Upper Anderson Creek (LAC, MAC, UAC), Lower and Upper Indian Creek (LIC, UIC) and Middle and Upper Rancheria Creek (MRC, URC) were experiencing temperature stress. Juvenile steelhead expressing high concentrations of hsp72, were consistently smaller than fish from cool water locations (unpublished data), but this potentially significant correlation is confounded by the lack of information on food availability and other factors at our field sites.

Asymmetry is used as an indicator of developmental stability in a broad range of animals and are typically expressed as variation between right and left (R-L) metric and meristic bilateral traits. Asymmetry is known to be a robust predictor of growth, survival ability,

and fecundity and has been negatively correlated with fitness in rainbow trout (*O. mykiss*. Asymmetry was examined at 13 of the 15 sites for the summer of 2000 (no fish were caught at the remaining two sites). Eight out of thirteen sites exhibited FA in meristic traits, 10 out of 13 sites exhibited FA in metric traits, and 12 out of 13 sites exhibited FA over all traits. These results indicate that fish in the very early stages (egg, alevin) may be exposed to stressors, most probably high water temperatures.

Finally, we looked at the interaction of two stressors, temperature and zinc by exposing fish to high water temperatures and dietary zinc in the laboratory. One of the primary results of this experiment was the lack of any statistically significant interaction between temperature and zinc. In fact, for no endpoint was the interaction even close to significant indicating that there are no synergistic effects between exposure to zinc and a moderate increase in temperature. Temperature clearly reduced growth rate, a common result in studies of this type. Increased zinc in diets caused changes in many experimental endpoints, some of them apparently confounding. For example, feeding rate was higher for fish exposed to increased zinc while growth under zinc exposure was lower than growth in control fish.

PREFACE

This report summarizes an investigation conducted from 1998-2002 on the effects of the two primary anthropogenic stressors, sediment and temperature, on salmonids in the Navarro River watershed. The Navarro River watershed currently has 13 designated and one potential beneficial uses. The primary beneficial uses of interest to this project are Migration of Aquatic Organisms including anadromous fish (MIGR), and habitat for Spawning, Reproduction, and/or Early Development of fish (SPWN). Both of these beneficial uses are presumed to be impaired as a result of excess sediment inputs and high water temperatures. Excess sediment causes a series of problems including smothering of redds of fish with the resultant failure of the recently hatched fish to emerge from the gravel and filling of pools used by fish during the latter part of the year. High water temperature causes both acute and chronic temperature stress in fish as well as decreases in dissolved oxygen. Both sediment and temperature have been implicated in the decline and extirpation of runs of salmonids across their entire range (e.g., NMFS 1996, Spence et al. 1996,Myrick and Cech 2001, Sullivan et al. 2000).

As a result of the impairment of the beneficial uses, the Clean Water Act requires the development of Total Maximum Daily Loads (TMDL) for those stressors responsible for the impairment. In the case of the Navarro River watershed, the North Coast Regional Water Quality Control Board developed TMDL standards aimed at restoring the river system to a state that will support the anadromous fisheries. Commissioned by the California Department of Transportation, this study addressed the following primary objectives: 1) determine the absolute and relative contribution of Caltrans to the stressor loads within the watershed, and 2) determine if the timing, magnitude, and duration of

these inputs were proportionately or disproportionately responsible for the decline of salmonids and the continuing degradation of salmonid habitat within the watershed. This document brings together the work from two related projects, the North Coast River Loading and Small Streams Crossing projects. The research conducted for the NCRL project addressed the first objective, and the SSC project addressed the second objective.

When these projects were conceived, many of the techniques needed to perform the research were not yet developed. Consequently, some of the work reported here represents the development of new methodologies for evaluating temperature and sediment loads. All of the research presented in this report has or will be submitted to peer-reviewed journals for publication. Much of the research initiated during this project is ongoing and the reader is encouraged to contact the authors of this report for updates over the next several months. Also, because of state and federal permitting restrictions, we were able to primarily study steelhead trout even though both steelhead and coho salmon are present in the watershed. Our take permits allowed us only to observe coho, and consequently our data for coho are restricted to spawning surveys and snorkel counts of juvenile fish.

This report is organized into three volumes. The first volume provides a basic discussion of the present status of fish in the watershed including the distribution, community structure, demographic status and genetic structure of the steelhead. The second volume provides a survey of the primary stressors in the watershed including sediment, temperature, contaminants in the water, and the effects of predation and competition.

The third volume discusses the impact of the stressors on the salmonids, specifically steelhead. Again, it is to be emphasized that many of these analyses are still in progress and will be completed over the next several months. However, due to changes in funding priorities and funds allocated by Caltrans, many projects that were initiated could not be completed, e.g., the impacts of watershed stressors on community structure and the resultant impacts on salmonids. Consequently, there remain several gaps in the analysis that prevent our being able to present a complete picture of the impacts of stressors on salmonids in the Navarro River watershed.

CURRENT STATUS

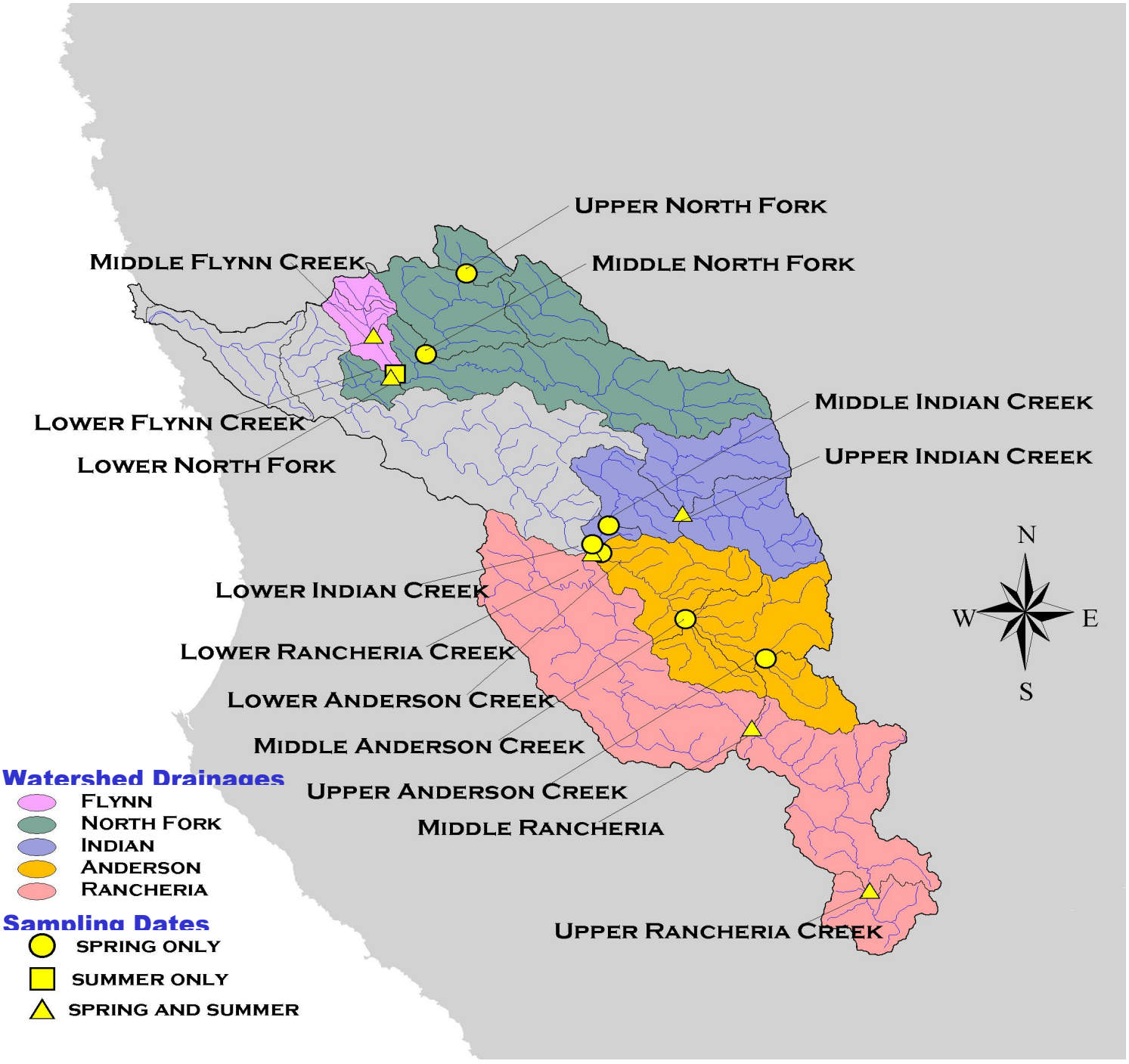
Watershed Description

The Navarro River watershed, located in Mendocino County, California, has a drainage area of 785 km^2 and drains to the Pacific Ocean just south of the town of Albion (Figure 1-1). The watershed is primarily forested with about 33% of the land in conifer forests while another 25% of the drainage area consists of hardwood rangeland (Figure 2). These hardwood areas are basically rangeland with at least 70% or greater canopy cover produced from hardwood tree cover (e.g. oaks) as indicated in remotely sensed images. The rest of the watershed consists of herbaceous and shrub land (24%), riparian (16%), and urban barren ground and other area (2%). Land use within the watershed consists mainly of logging, grazing, farming and vineyard activities. The Navarro watershed studies focused on five streams and their watersheds: Rancheria Creek, Anderson Creek, Indian Creek, North Fork, and Flynn Creek. All five sub-watersheds drain directly to the narrow floodplain of the Navarro River that runs next to Highway 128. Within each of the study drainages research activities were generally confined to each of three stream segments located in the upper, middle and lower portion of each watershed.

Current status and distribution of anadromous fish

Two species of anadromous fish are currently found in the Navarro River watershed, coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*Oncorhynchus mykiss*). The North Fork is the westernmost watershed and is primarily second/third growth redwood and Douglas fir and is included in the fog belt that moves in from the Pacific Ocean. Historical distribution of the coho covered 130 miles of the Navarro and its tributaries as

Figure 1-1. Map of the Navarro River watershed in Mendocino County. The yellow circles, squares, and triangles represent sampling locations within each of the subwatersheds. The gray area within the dark outline of the watershed is the mainstem of the Navarro.



of 1963, and extended to Flynn Creek and Indian Creek, the two watersheds to the immediate west of the North Fork (Moyle and Brown 1991). During a CDFG survey (CDFG 2001), coho were present only in the North Fork and its tributaries and Flynn Creek. Using Geographic Information Systems, we identified a 78.4 percent reduction in the linear distribution of coho salmon from their historic watershed distribution over the last 12 years.

Steelhead, the anadromous form of the rainbow trout, once ranged along the eastern Pacific coast from Alaska to Baja California. Currently all three recognized forms of steelhead in California are considered to be in decline (winter steelhead) and qualify as threatened (summer steelhead) or endangered (southern steelhead) under the federal Endangered Species Act (Moyle et al. 1995). Significant threats to the continued survival of genetically distinct California steelhead populations include habitat degradation and loss, competition with and predation by introduced fishes, and introgressive hybridization with hatchery rainbow trout (Williams et al. 1989, Moyle et al. 1995).

Other common fish species in the watershed include: California roach (*Lavinia symmetricus navarroensis*), three-spined stickleback (*Gasterosteus aculeatus aculeatus*), Sacramento sucker (*Catostomus occidentalis*), coast range sculpin (*Cottus aleuticus*), prickly sculpin (*Cottus asper*), pacific brook lamprey (*Lampetra pacifica*), and pacific lamprey (*Lampetra tridentata*). The California roach is the most common species of fish throughout the watershed.

Determination of anadromous status of *O. mykiss*

Coho are restricted to the North Fork and Flynn Creek watersheds (but see spawning below), but steelhead occupy all of the main tributaries. However, steelhead are the anadromous form of the rainbow trout, and it is possible that the fish in various portions of the watershed are not anadromous or that both anadromous and resident forms coexist, either sympatrically or allopatrically. Several salmonids including rainbow trout, coastal cutthroat, sockeye, brown trout, and Arctic charr may exhibit both life history traits (Zimmerman and Reeves 2000). It is possible to distinguish these two types fish using otolith microchemistry, specifically the ratio of strontium to calcium in the core and in the outer edges of the otolith. If the majority (or all) of the *O. mykiss* in the watershed were of resident origin, restoration of the anadromous steelhead would involve recolonization of the watershed and the potential would exist that the TMDL load restrictions would not apply. Consequently, we undertook an investigation to determine if the *O. mykiss* in the watershed are steelhead or resident rainbow trout.

Sr:Ca analysis

Otoliths (earstones) are biogenic, calcareous concretions that serve as part of the hearing and balance (acoustico-laterelis) system in fishes. They consist of calcium carbonate (usual form is aragonite) precipitated in a protein matrix (endolymph) and reside in the semi-circular canals under the braincase (Degens et al. 1969). Fish contain three different pairs of otoliths: the sagittae within the sacculus, the asteriscus within the lagena, and the lapillus within the ultriculus. Usually the sagitta are the most developed earstone of the fish and, henceforth, the most often studied. Otolith growth is a one-way, metabolically inert process. New acellular material is added to the outside surface through time but existing material cannot be removed. This one-way process results in

the formation of daily rings, which provides a visible history of the fish's age and growth. The fact that the otolith is acellular and metabolically inert means that any elements or compounds accreted onto its growing surface are permanently retained as an environmental recorder of the fish's exposure to its chemical environment (Campana and Neilson 1985).

Analysis of otolith microchemistry, particularly strontium to calcium ratios (Sr:Ca), provides a means of detecting levels of anadromy in fish populations (Kalish 1990, Secor 1992, Halden et al. 1995, 1996, Limburg 1995, Secor et al. 1995, Tzeng et al. 1997, Otake and Uchida 1998, Tsukamoto et al. 1998, Zimmerman and Reeves 2000). Strontium is substituted for calcium in the otolith carbonate matrix at levels relative to environmental concentrations. Each 1% increase in salinity produces a 0.05×10^{-3} increase in otolith Sr:Ca molar ratio. Given the 30 to 35% difference in salinities between the marine environment and riverine waters, this corresponds to a 1.5×10^{-3} change (or threefold) in the otolith Sr:Ca ratio (Campana 1999). The ratio of Sr:Ca is measured rather than the absolute concentration of each dissolved element because the branchial uptake of metals generally decreases as the relative concentration of calcium in the water increases (Mayer et al. 1994). Comparison of Sr:Ca ratios in the primordia (cores) and exogenous-feeding freshwater growth regions can be used to determine maternal origin (resident or anadromous), based on the assumption that primordium composition reflects the environment in which yolk precursors develop (in the ocean for anadromous forms) (Kalish 1990).

Otolith collection, preparation and microchemical analysis

Sagittae of juvenile summer steelhead were collected from six tributaries of the Navarro River between May and August of 2000. When possible, samples were collected from the upper, middle, and lower sections of each tributary. Logistical constraints restricted sampling of John Smith Creek to a single location and Flynn Creek to a lower and middle location. No smolts from the estuary were examined. Samples were collected with bag seines or by electrofishing and immediately placed on dry ice before being transported to the laboratory where they were placed in the -80ºC freezer for several weeks (late summer fish) to a couple of months (early summer fish).

Otolith extraction and preparation

Sagittal otoliths were removed from the fish, soaked in water, rubbed clean of excess tissue, and air-dried. Borosilicate glass rings (25 mm diam.) were cut with a 6" diamond saw to a thickness of approximately 5 mm for mounting on the petrographic slides. The glass rings were polished flat on one side with a Jarvi Tool Facetron diamond lap with a 260-grit diamond disk. The polished glass rings were rinsed and then cleaned in a Cole-Parmer ultrasonic cleaner with tap water for 30 seconds to remove any grit. The glass rings provided a protective barrier against loss of edge material while grinding and polishing the otoliths. The slides and glass rings were placed on a sheet of paper and heated to 121°C on a hotplate. A thin layer of Petropoxy 154 glue was applied to the polished side of a ring. The ring was then centered on a slide and allowed to dry for a minimum of 20 minutes on the hotplate. This process was repeated for each slide. An Ingram thin section grinder with a 320 grit diamond wheel was used to grind each slide-

mounted glass ring to a thickness of 2 millimeters. After grinding the slides were rinsed and then cleaned in the ultrasonic cleaner with tap water for 30 seconds to remove any grit. After removal from the ultrasonic cleaner the slides were rinsed again and dried on a hotplate. When the slides had reached 121°C four otoliths were placed sulcus side down on each slide. The otolith identification and position on the slide were recorded and a thin bamboo skewer was used to apply a fine bead of Petropoxy 154 around the perimeter of each otolith. After 20 minutes on the hotplate a thin layer of Petropoxy 154 was applied across the surface of each otolith and allowed to dry for another 20 minutes so that the epoxy completely embedded the otolith.

One slide at a time was placed in an Ingram thin section grinder with a 320 grit diamond wheel and ground until contact was made with the surface of the each otolith. The process was repeated for each slide. This procedure required constant microscopic examination of the otoliths to prevent over-grinding. After grinding the slides were rinsed and then cleaned in the ultrasonic cleaner with tap water for 30 seconds to remove any polish residue. Each slide was then placed in a custom plexiglass holder and hand polished one at a time on a glass plate with $A1_2O_3$ (3 μm width) using a circular motion and light pressure for approximately 15 seconds. After polishing on the glass plate the slides and plexiglass holder were rinsed and then cleaned in the ultrasonic cleaner with tap water for 30 seconds to remove any polish residue. For the final polishing each slide was again placed in the custom plexiglass holder and hand polished, one at a time, with silk cloth and 0.05 μm width $A1_2O_3$ on a Buehler Ecomet III grinder/polisher for approximately 20 seconds. All otoliths were then examined under a microscope and the

entire polishing process was repeated if the thickness of an otolith was too great to prevent viewing of the primordia and pre-hatch growth rings. After final polishing the slides were rinsed and then cleaned in the ultrasonic cleaner with tap water for 30 seconds to remove any polish residue. The slide containing several otoliths was cleaned with acetone, air-dried, and coated with a 250-Å carbon layer (1 Å = 0.1nm).

Microchemical Analysis

Strontium and calcium analyses were performed with a Cameca SX-50 wavelength dispersive electron microprobe. Probe current and accelerating voltage were 45-nA and 15-kV, respectively. A 10 μm diameter beam was used for all analyses. Strontium sulfate ($SrSO_4$) and calcite ($CaCO_3$ (USNM 136321)) were used as standards for strontium and calcium, respectively. Strontium was analyzed for 60 s for background counts and for 120 s for peak counts. Calcium was analyzed for 8 s for background counts and for 16 s for peak counts. The average counts from these two background measurements were subtracted from the peak. Strontium was measured using the TAP crystal and calcium was measured using the PET crystal.

Otolith regions were defined as maternally influenced growth regions (primordia) and exogenous-feeding freshwater growth regions uinfluenced by yolksac absorption (i.e. outside the nucleus). As many primordia were analyzed as possible, ranging from one to several. Many cracks formed in the otoliths as a result of epoxy drying and often times these cracks extended through the cores, which prevented their analasis. Three evenly spaced transect measurements were made outside the primordia up to 50 μm from the

edge of the epoxy at the perimeter of the otolith. Two rim measurements were made 20 μm from the edge of the epoxy (Figure 1-2). All measurements made outside the nucleii are assumed to derive from freshwater growth since all fish are 0+ in age. The average distance between primordia (when multiple primordia were available for analysis) was 78 μm and the average distance from the first primordia read to the rim measurements was 373 micrometers. Maternal origin for each fish was determined by comparing mean Sr:Ca ratios in the primordia with mean Sr:Ca ratios in the region outside the nucleus. A fish was determined to be of anadromous maternal origin if the averaged primordial Sr:Ca ratio was significantly greater than the averaged transect and rim Sr:Ca measurements. Results were considered significant based on a paired one-tailed *t*-test with $\alpha = 0.05$.

Results

The only exogenous-feeding freshwater growth measurements included in the analyses were from rims and transect measurements greater than 85 μm from the first primordia. Kalish (1990) found that the nucleus length for a single *O. mykiss* fry of sea-farmed

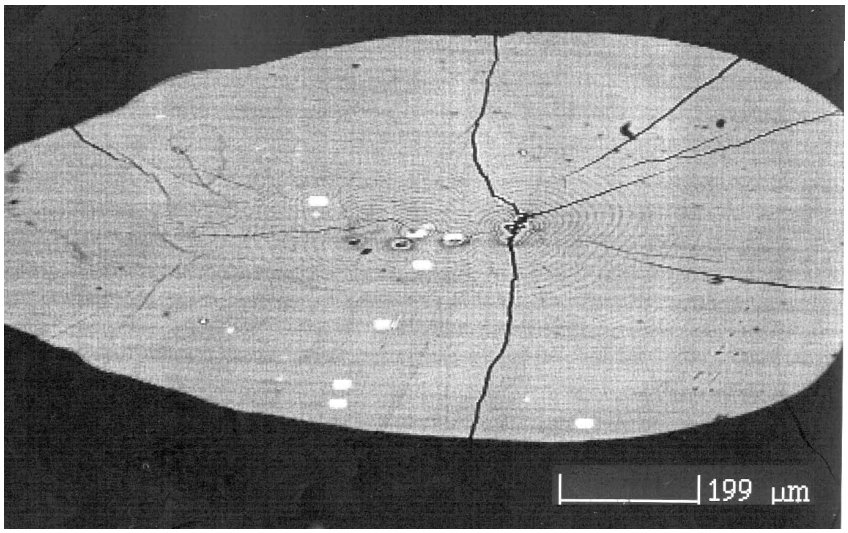


Figure 1-2: Backscatter electron image (BSE) of sagitta for sample taken from Upper Indian Creek on June 7, 2000. Daily rings are visible in addition to x-ray burn marks for three primordia, three transect measurements, and two rim measurements. Cracking is a result of epoxy drying (mag = 70X).

broodstock origin was 170 μm so transect measurements less than 85 μm from the core were not included in the analyses because they might have been read within the range of the nucleus. A frequency distribution curve for the highest possible values (based on the error that is inherent in x-ray analysis) of the mean primordial Sr:Ca ratios minus the mean transect and rim Sr:Ca ratios can be found in Figure 2. Of the 143 juvenile *O. mykiss* sagittae analyzed, all had significantly higher measured Sr:Ca ratios in the primordia than in the exogenous-feeding freshwater growth ($t = 14.86$, $P < 0.0000$). The results are normally distributed with a standard error for the averaged primordia and transect/rim Sr:Ca ratios of 4.76×10^{-2} and 2.98×10^{-2} respectively. A one-way test of variance (ANOVA) between tributaries was performed for the measured cores, the measured transects and rims, and the measured difference between the core and outside-core measurements. No significant variance was observed for the Sr:Ca measurements made in the cores. However, there was significant variability for Sr:Ca measurements made in the freshwater growth region ($P < .05$; F-ratio = 3.06; DF = 4 & 138) and the difference between the core and outside core measurements ($P < .05$; F-ratio = 2.50; DF = 4 & 138). For both tests where variability was observed, Indian Creek was different from the North Fork.

Discussion

Given the positive values for the differences between the highest possible core and rim Sr:Ca ratios and the unimodal frequency distribution cure (Figure 2), we cannot conclude that any fish in our sample were of maternal freshwater resident origin. A fish from

Lower Indian Creek taken on May 24, 2000 and another from Lower Rancheria Creek taken on June 7, 2000 have differences of 0.1389 and 0.0028, respectively, and could

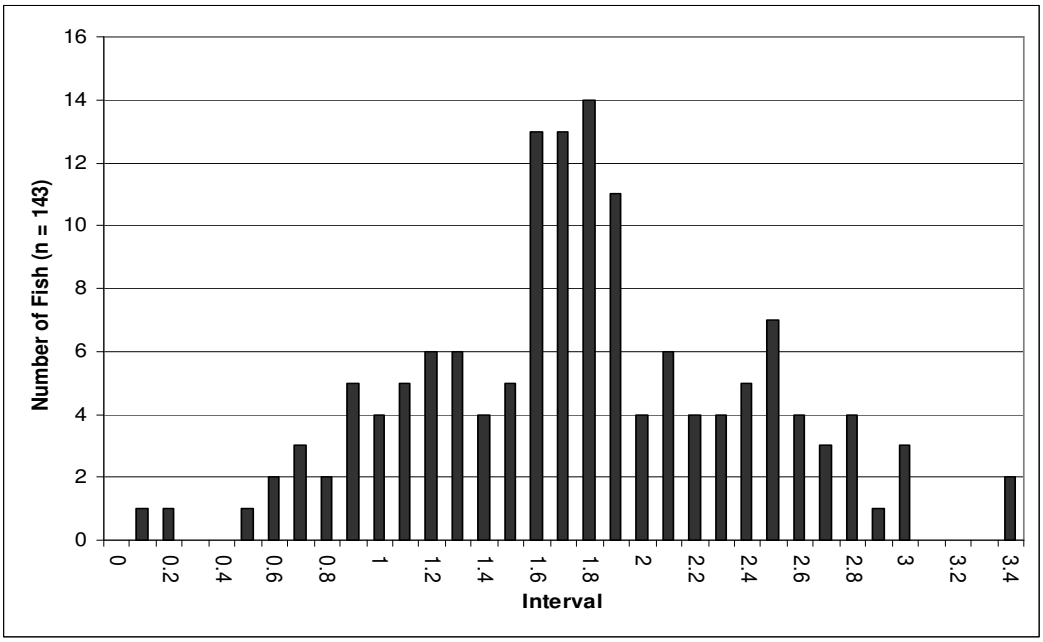
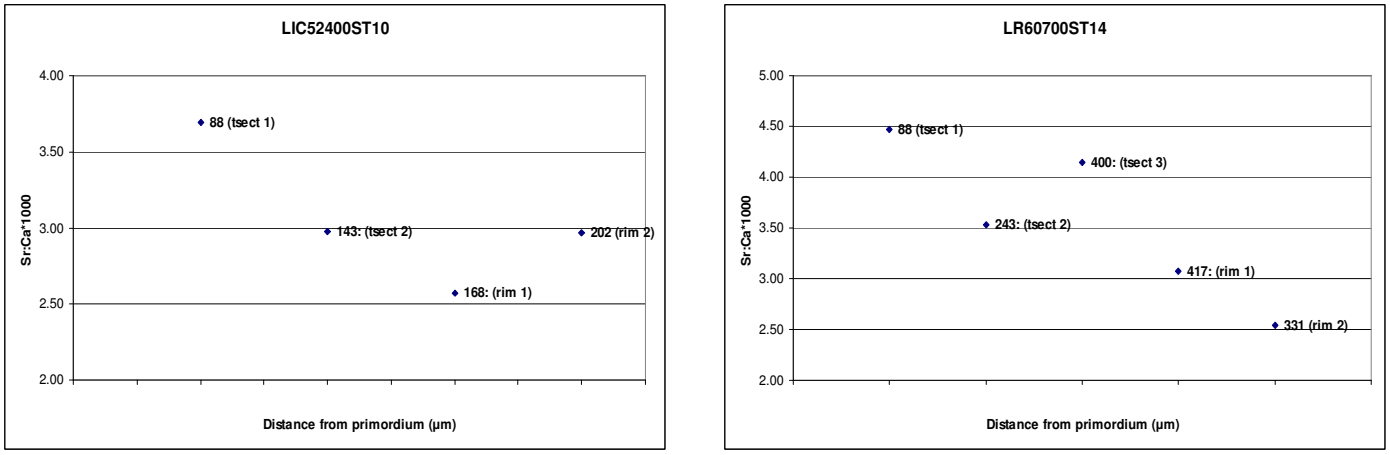


Figure 1-3: Frequency distribution curve of the highest possible values for the mean core Sr:Ca ratio measurements minus the mean transect and rim Sr:Ca ratio measurements (ratios are multiplied by 1000).

a. **b.**



Figures 1-4a, b: Sr:Ca measurements for samples taken from Lower Indian Creek on May 24, 2000 and Lower Rancheria Creek on June 7, 2000. Exact distances from the core can be found next to each data point. Note the decreasing Sr:Ca ratio from transect no. 1 to rim measurements.

possibly be considered the offspring of freshwater residents. However, the first transect measurements made for both fish were only 88 µm from the core. There is a strong chance that these measurements could have been made within range of the nucleus since our presumption for an 85 µm nuclear radius is based on only one fish (Kalish 1990). When the data are plotted (Figures 3a and 3b) one can observe a decreasing trend in the Sr:Ca ratio from the first transect measurement to the rim measurements, indicating the possibility of incomplete yolk sac absorption by the first transect measurement. Furthermore, the fact that variance tests yielded no significant differences between tributaries for core measurements but did reveal significant differences in freshwater growth regions confirms our belief that all the fish in our sample result from anadromous mothers.

There is only an average 1.26-fold change in the measured Sr:Ca ratio between the primordia and freshwater-growth regions in our sample, not the expected threefold change. The Sr:Ca ratio of seawater is approximately 8.74 X 10^{-3} (Bruland 1983). This is considerably higher than the core measurements (mean = 3.69 X 10^{-3}) and exactly three times higher than the freshwater measurements (mean = 2.92 X 10^{-3}) made for our sample, which suggests an attenuation of the primordial Sr:Ca signal. Furthermore, the broad frequency distribution observed in Figure 2 (range of difference: 2.78 X 10^{-6} to 3.40 X 10^{-3}) suggests a modification of varying degrees to the primordial Sr:Ca ratios.

A variety of environmental, chemical, and stress factors may influence the mobilization and availability of calcium and strontium in the yolk proteins of the developing ova and, ultimately, in the substitution of strontium for calcium in the carbonate matrix of the offspring's primordia (Kalish 1989). There is some evidence that ion exchange can occur between the female and the egg during the period prior to spawning (Alderdice 1988). It is possible that time spent by the pre-spawning mother in the estuary dilutes the strontium signal in the eggs to a degree reflecting estuarine residence. Maybe some of the female steelheads reside in the river plume portion of the ocean during egg formation so less strontium than expected is sequestered in the yolk sac Otolith Sr:Ca ratios have also been shown to vary with temperature (Radtke 1989, Townsend et al. 1992, 1995, Secor et al. 1995). Townsend et al. (1992) suggest that physiological processes for Atlantic Herring become slowed and impaired at lower temperatures so strontium is allowed to pass more readily into the endolymph and become incorporated into the otolith aragonite. Perhaps other physiological effects like growth rate or stress alter the maternal uptake of

strontium and calcium during egg formation. Secor et al. (1995) found that striped bass growth rates significantly effected otolith Sr:Ca ratios. Kalish (1992) attributed increases of Sr:Ca ratios at the edge of Australian salmon otoliths to a stress-induced incorporation of increased strontium levels by the fish. Furthermore, dissolved oxygen concentration and pH can also influence the elemental uptake into the fish (Mayer et al. 1994). The spatial and temporal variability of these environmental factors during egg production could result in the broad distribution of core and outside-core Sr:Ca ratio differences. We are currently examining the relationship between temperature, growth rate, and Sr:Ca ratios to determine if any of these factors could be responsible for the range of Sr:Ca values seen in the otoliths of juvenile fish. A lack of any relationship would lead to the conclusion that estuarine residence of the pre-spawning mother was a factor in the range of Sr:Ca values observed, and point to the importance of estuarine residence of females prior to initiating the spawning run to the natal watershed.

This analysis is ongoing and we anticipate being able to answer these questions within a few weeks. Readers are encouraged to contact the authors for final results of this analysis.

Demographic Analyses

Navarro spawning ground/carcass survey protocol

Between 15 December 1999 and 14 April 2002 the Navarro River and tributaries were surveyed to determine the presence and abundance of spawning steelhead trout (*O. mykiss*) and coho salmon (*O. kisutch*). The surveys were performed between early November and late May to encompass the period of adult salmonid activity in the Navarro watershed. Survey dates coincided with times of adequate winter storm run-off since this is the primary factor that influences spawner entrance to the tributaries. All reaches were surveyed three times during the spawning season (following the first major winter storm/estuary breeching event, peak-spawn and post-spawn) pending personnel availability. Survey reaches were selected primarily on 1) overlap with existing study areas within the watershed 2) areas of known salmonid presence based on previous studies and 3) areas where we could lawfully access the waterways.

Survey Reaches

RANCHERIA CREEK:

- **Lower --** from the confluence with Indian Creek upstream approximately 2.5 miles to landslide.
- **Middle** -- from Ornbaum Creek upstream to Fish Rock Road.
- **Upper** -- from Humboldt State University road crossing approximately 1.5 miles downstream of Foppiano bridge on Elk Horn Road to upstream of Foppiano bridge approximately 1.5 miles at Bickell Ranch gate.

ANDERSON CREEK:

- **Lower** -- from confluence with Rancheria Creek upstream to Hwy 128 bridge at Boonville (encompassed both lower and middle snorkeling units).
- **Upper** -- from Hwy 253 bridge upstream approximately 3 miles to road crossing upstream of confluence with Jimmy Creek.

INDIAN CREEK:

- **Lower** --from confluence with Rancheria Creek upstream approximately 3 miles to old skid road on southeast side of creek (encompassed both lower and middle snorkeling units).
- **Upper --** from first road crossing on Lebieu property off Peachland Road upstream to North Fork of Indian Creek and then upstream on North Fork Indian Creek approximately one mile to the next road crossing.

NORTH FORK OF NAVARRO RIVER:

- **Lower** -- From Scale Ramp Road on Hwy 128 upstream to the Hwy 128 bridge at Masonite Road.

- **Middle --** From the Hwy 128 bridge at Masonite Road upstream to the confluence with Dutch Henry Creek.
- **Upper** -- From the confluence with Dutch Henry Crk upstream to the confluence with Redwood Creek.

  Supplementary:
  - The South Branch of the North Fork from the confluence with the North Fork upstream for approximately 1.5 miles.
  - John Smith Creek from the confluence with the North Fork upstream for approximately 2 miles.

FLYNN CREEK

- From the confluence with the North Fork upstream approximately 4 miles (Flynn Crk. Rd X-ing)

COASTAL GULCHES

- **Flume Gulch** -- from the confluence with the Navarro R. upstream for approximately 1.5 miles.
- **Marsh Gulch** -- from the confluence with the Navarro R. upstream for approximately 1.5 miles.
- **Murray Gulch** -- from the confluence with the Navarro R. upstream for approximately 1.5 miles.
- **Ray Gulch** -- from the confluence with the Navarro R. upstream for approximately 1 mile.
- **Barton Gulch** -- from the confluence with the Navarro R. upstream for approximately 1 mile.
- **Mustard Gulch** -- from the confluence with the Navarro R. upstream for approximately 1mile.

Redd Survey Methods

Stream reaches were surveyed while hiking upstream. Salmonid redds were identified as areas of cleaned and sorted gravels (20mm-100mm) with a clearly defined pit and tailspill, or any area where fish were observed spawning. Salmonid redds were usually located in tailout areas of pools and runs

Redd Survey Results

Survey results (Table 1-1 and Figures 1-5 and 1-6) are still in the process of being analyzed and only a few results are presented here. Spawning occurred in all three years surveyed. Interestingly, there were as many spawning steelhead in Anderson and Indian Creeks as in the North Fork in what is considered prime spawning habitat. As would be expected based on the general amount of rainfall over the three seasons, the winter of 2000-01 was the best year for both species, and the winter of 2001-02 was the worst year. The winter of 2001-02 was characterized by high rainfall amounts early in the season, and then almost a complete lack of rain until late in the winter. As a result, only the early

spawning coho and the late spawning steelhead were able to be successful in 2001-02 (no surveys of Rancheria Creek were conducted due to funding constraints).

If we assume that most of the returning spawning females are between 1-2 kg, and their egg production is approximately 2000 eggs/kg of body weight, the minimum number of eggs produced in the Navarro watershed per year range from 65,000/130,000 eggs in 2001-02 to 298,000/586,000 eggs in 2000-01.

Table 1-1. Summary of spawning surveys conducted during the three years of the project. Results were not standardized for unit effort or distance covered. A "0" value indicates that the area was surveyed but no redds were located. The absence of a data label indicates that the location was not surveyed. Locations of redds were recorded with a GPS unit but are not provided in this report.

Date Begin	Date End	No. Sample Days	Tributary	Avg Temp(C)	WST Count	COHO Count	UNKNOWN Count	FishOn
1999-2000 Spawning season								
3/31/2000	4/26/2000	4	ANDERSON CREEK		44	0	0	19
12/15/1999	12/15/2000	4	FLYNN CREEK		0	7	0	0
3/30/2000	4/26/2000	3	INDIAN CREEK		17	0	0	3
4/5/2000	12/5/2000	5	NORTH FORK		19	4	0	2
3/31/2000	4/6/2000	2	RANCHERIA CREEK		6	0	0	4
4/22/2000	4/22/2000	1	NAVARRO MAINSTEM		14	0	0	0
			BARTON GULCH					
2000-2001 Spawning season								
12/16/2000	12/16/2000	1	MARSH GULCH		0	0	0	
12/16/2000	12/16/2000	1	MURRAY GULCH		0	0	0	
			RAY GULCH					
3/15/2001	4/18/2001	4	ANDERSON CREEK	10.3	34	0	0	12
1/4/2001	4/2/2001	4	FLYNN CREEK	5.5	2	8	0	2+
3/20/2001	4/19/2001	4	INDIAN CREEK	11.9	38	0	0	15+
1/5/2001	4/11/2001	11	NORTH FORK	7.0	47	20	17	13+
3/9/2001	4/18/2001	5	RANCHERIA CREEK	11.3	21	0	0	0
1/5/2001	1/30/2001	2	NAVARRO MAINSTEM	6.5	0	0	0	
4/4/2001	4/4/2001	1	BARTON GULCH		1	0	0	
2/16/2001	4/4/2001	3	MARSH GULCH	7.7	3	0	0	
3/22/2001	4/4/2001	2	MURRAY GULCH	9.5	2	0	0	
3/8/2001	4/4/2001	2	RAY GULCH		2	0	0	
2001-2002 Spawning season								
3/26/2002	3/26/2002	1	ANDERSON CREEK	11.0	11	0	0	2
1/20/2002	1/20/2002	1	FLYNN CREEK	8.0	0	3	0	0
3/26/2002	4/14/2002	3	INDIAN CREEK	14.3	17	1	0	0
1/27/2002	4/13/2002	3	NORTH FORK	13.7	9	1	0	0
			RANCHERIA CREEK					
			NAVARRO MAINSTEM					
			BARTON GULCH					
			MARSH GULCH					
			MURRAY GULCH					
			RAY GULCH					

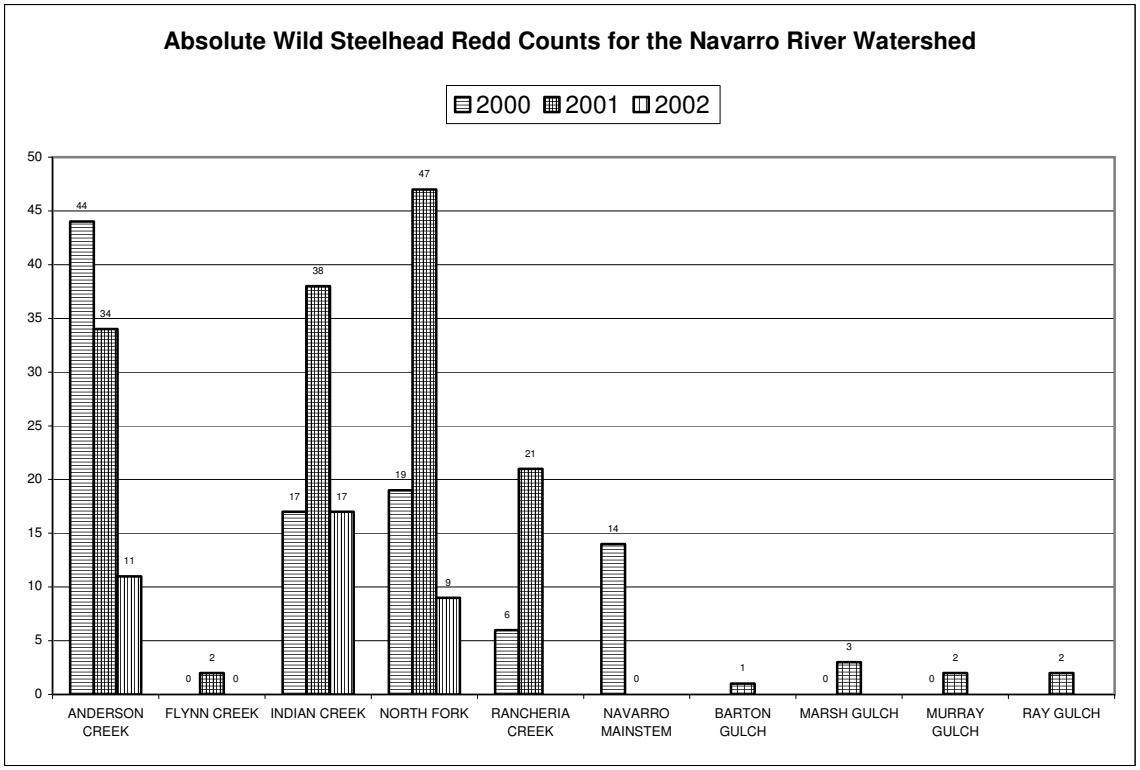


Figure 1-5. Numbers of steelhead redds counted during the years 1999-00, 2001-02. Numbers above the histogram bars indicate the number observed. No number above the axis indicates counts were not made, the number 0 above the x-axis indicates the section was walked but no redds were found. All redds were located with GPS coordinates to prevent double counting.

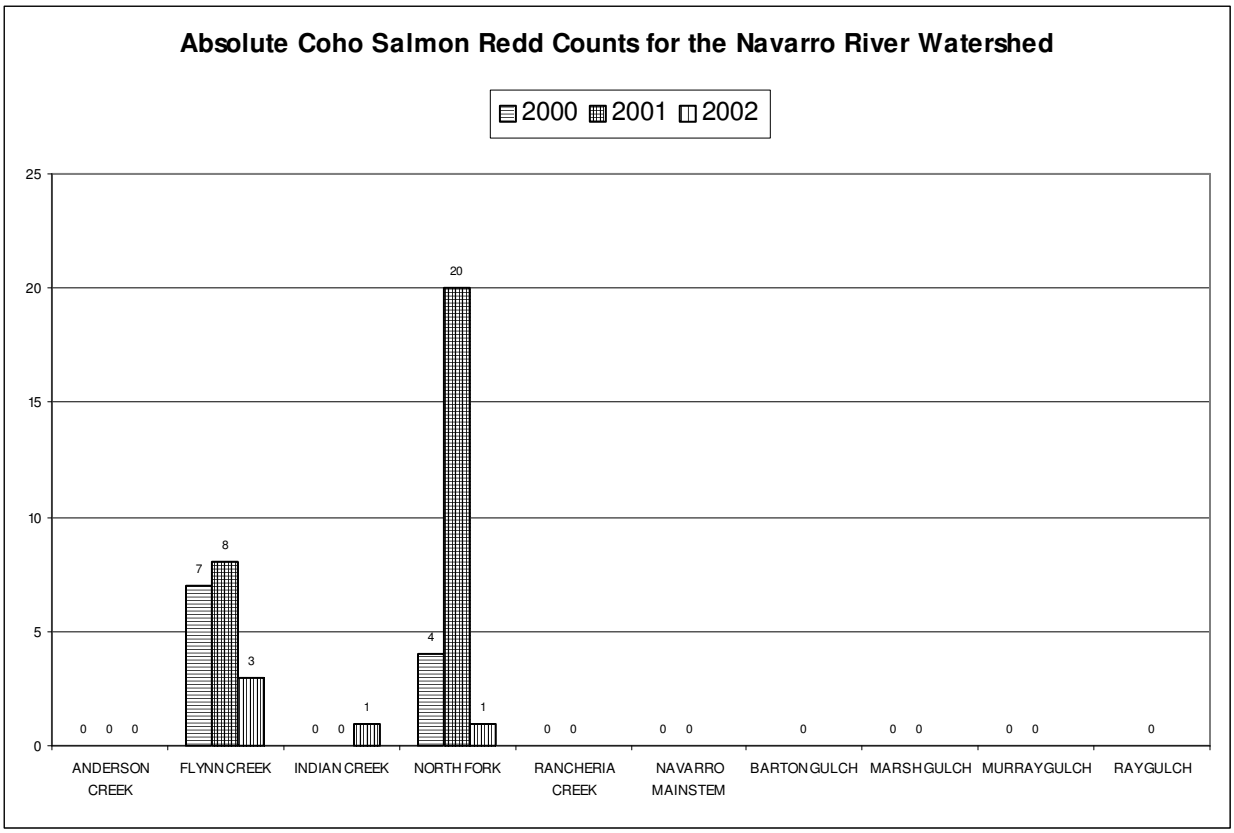


Figure 1-6. Numbers of coho salmon redds counted during the years 1999-00, 2001-02. Numbers above the histogram bars indicate the number observed. No number above the axis indicates counts were not made, the number 0 above the x-axis indicates the section was walked but no redds were found. All redds were located with GPS coordinates to prevent double counting.

Population estimates - Juvenile steelhead

Snorkel counts were performed using the protocol outlined in Hankin and Reeves (1988). Each site to be snorkeled contained at least three riffles and three pools. Beginning at the riffle or pool that delineated the downstream end of each site, and moving upstream, the habitat units were defined as being either riffle, pool, run or glide. Units other than the first three riffles and the first three pools at each site were deemed "supplementary" units. Once a minimum of three riffles and three pools had been counted the upstream end of the third riffle or third pool (whichever came last) was used to delineate the upstream boundary of that site.

Before snorkeling at each site a 30' beach seine of ¼" mesh was placed at the upstream end of the first habitat unit (e.g. riffle or pool) in order to prevent movement of fish into or out of the unit. Snorkelers were timed with a stop watch as the swam or crawled upstream counting all fish and amphibians in a habitat unit and verbally relaying their counts, through their snorkels, to a data recorder who was walking up the stream behind them. Snorkelers attempted to keep their heads underwater at all times so that movement of fish could be accounted for. Steelhead (*O. mykiss*) were recorded as either 0+ (young of the year) or 1+ (one year or older) based on obvious size differences.

If the unit to be snorkeled was determined to be too wide for one snorkeler to effectively cover then two snorkelers were used. When two snorkelers were counting in the same unit an imaginary line divided the center of the unit and each snorkeler only counted the fish between the imaginary center line and the stream bank nearest that snorkeler. Verbal communication and hand signals were used between snorkelers to identify when fish that

one snorkeler had already counted moved into the counting lane of the other snorkeler so that individual fish would not be counted more than once. Snorkelers moved upstream at the same rate as each other in order to minimize movement of fish.

When the snorkeler(s) reached the end of a habitat unit the number of snorkelers and time elapsed was recorded to account for sampling effort. After snorkeling each unit, the beach seine was then moved to the upstream end of the next unit and the entire process was repeated until all habitat units within the site had been snorkeled. After snorkeling the last unit stream temperature and time were recorded.

After all habitat units at a site had been snorkeled, the length and 3-5 widths for each habitat unit were measured. Lengths were measured along the thalweg while widths were recorded at the upstream, middle and downstream ends of the habitat unit using the wetted edge of the channel to define the boundaries. As many as five widths were measured in evenly spaced intervals at habitat units deemed to be unusually long. A maximum water depth was recorded in each habitat unit.

Results presented here are very preliminary and data are still being analyzed. As was the case for spawning, there were more juvenile fish present in the watershed during the summer of 2001 compared to 2000 (Table 1-2). The most productive subwatershed was Rancheria Creek followed by Indian Creek, Anderson Creek, North Fork, and Flynn Creeks. In the year 2000, only three sites (lower Flynn, upper Flynn, and lower North Fork) gained individuals from spring to summer samples (Figures 1-7 – 1-14). In 2001, there was an increase in the number of fish at two sites (upper Flynn, and lower North

Fork). Since the majority of the fish had emerged prior to the spring sampling period, the increase in numbers is a result of immigration to the site. In general however, there was a substantial loss of individuals from the subwatersheds in both years (Figures 1-7 – 1-14). The greatest losses occurred in Rancheria Creek and Indian Creek, two watersheds that are located inland and experience higher water temperatures. Interestingly, Anderson Creek, which is also located inland and experiences higher water temperatures lost relatively few fish. Further analyses will be completed over the next few months.

Emergence and growth rates

Otoliths were used to obtain growth rates for fish collected during both summers. Currently, otoliths from 343 fish from the summer of 2000 have been photographed, and the daily growth rings delineated and measured. Otoliths from the summer of 2001 are still being processed. Briefly, sagittae from each juvenile fish were removed from the fish (see above). One was polished lightly to enable the rings to be delinaeated. Otoliths were mounted and images were created, manipulated and measurements taken using Spot® RT Software (Diagnostic Instruments, version 3.0, 1999). Measurements were made by two different observers and reconciled if the estimated ages of the fish differed by more than 10%. The emergence of the fish from the gravel is identified by the location of a darker line near the primordium of the otolith. This darker line often obscures the growth lines that are laid down immediately after the emergence leading to most of the discrepancies.

At time of collection, the fork length of the fish is recorded. Using the fork length and an estimate of fish age, it is possible to calculate the fork length at any day in the life of the

fish using the procedure of Morita and Matsuishi (2001). Fork lengths for each fish for each day of its life were calculated, and the growth rates were calculated by simply taking the difference in fork length between adjacent days. An instantaneous growth rate will be calculated by taking the first derivative of fork length with respect to age according to equation 15 in Morita and Matsuishi (2001).

The earliest emergence date in 2000 was March 3, and the latest emergence date was June 16 (Figure 1-15). The bulk of the fish emerged during April. There appeared to be no pattern in emergence with respect to sites within watersheds, in general fish emerged at each site across the entire period of emergence.

When examined across all subwatersheds, growth rates were generally normally distributed with a mean daily growth rate of .51 mm/day (± .22mm/day StDev). Mean growth rate was negatively correlated with fish age ($r = -.40$, $df = 342$, $p = 0.000$) indicating that the older the fish, the slower the growth. This might be expected as fish rely on drift for their primary source of food, and flows within each of the subwatersheds decline as the summer progresses. Fish often become stranded in pools and have to rely on insects falling directly into the pools. Shifting their diet to the available benthic invertebrates would result in a depletion of that food resource and a reduction in available energy. In addition, as the fish experience temperature stress they produce heat shock proteins, which can be a substantial energetic demand (Volume 3). There is essentially no correlation of growth rate with emergence date ($r = 0.09$, $df = 342$, $p = 0.10$)

indicating that fish emerging earlier did not grow any faster than fish emerging later in the year.

When examined on a watershed basis (Table 1-2), there are substantial differences in the relationships between growth rate and age and date of emergence. In the North Fork and Flynn Creek, there is no relationship between growth rate and age or emergence date (NF: age – $r = -0.13$, $df = 71$, $p = 0.30$; emergence – $r = 0.07$, $df = 71$, $p = 0.57$, FC: age – $r = -.04$, $df = 30$, $p = 0.822$; emergence – $r = -.13$, $df = 30$, $p = 0.363$). In Anderson Creek, growth rate is negatively correlated with both age and emergence date (age – $r = -.42$, $df = 79$, $p = 0.0001$; emergence – $r = -.38$, $df = 79$, $p = 0.0006$) and in Indian Creek, the correlation with age is negative ($r = -.52$, $df = 78$, $p = 0.0000$) and positive with emergence date ($r = .37$, $df = 78$, $p = 0.0009$). In Rancheria Creek, the correlation of growth with age is negative ($r = -.48$, $df = 84$, $p = 0.0000$) and positive with emergence date ($r = .22$, $df = 84$, $p = 0.037$). The correlation of growth rate with age is always negative, although not always significantly. The correlation with emergence date is positive, negative, or nonexistent indicating that site-specific factors are important in setting growth rates.

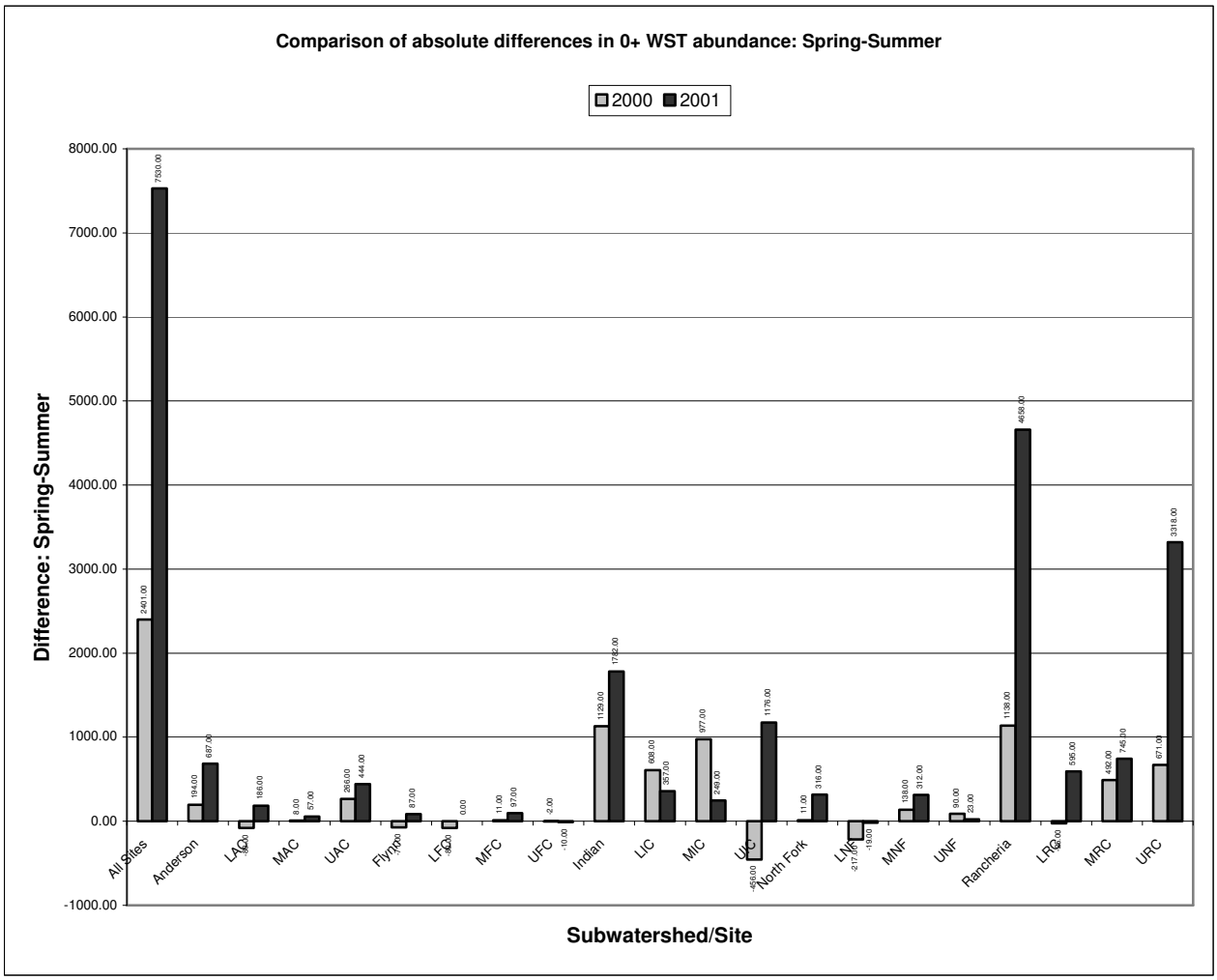


Figure 1-7. Differences between the spring and summer sampling periods by site and watershed in the number of 0+ steelhead. Positive numbers indicate that there were more fish in the spring than the summer. Negative numbers indicate more fish in the summer with the increase being the result of immigration to the site.

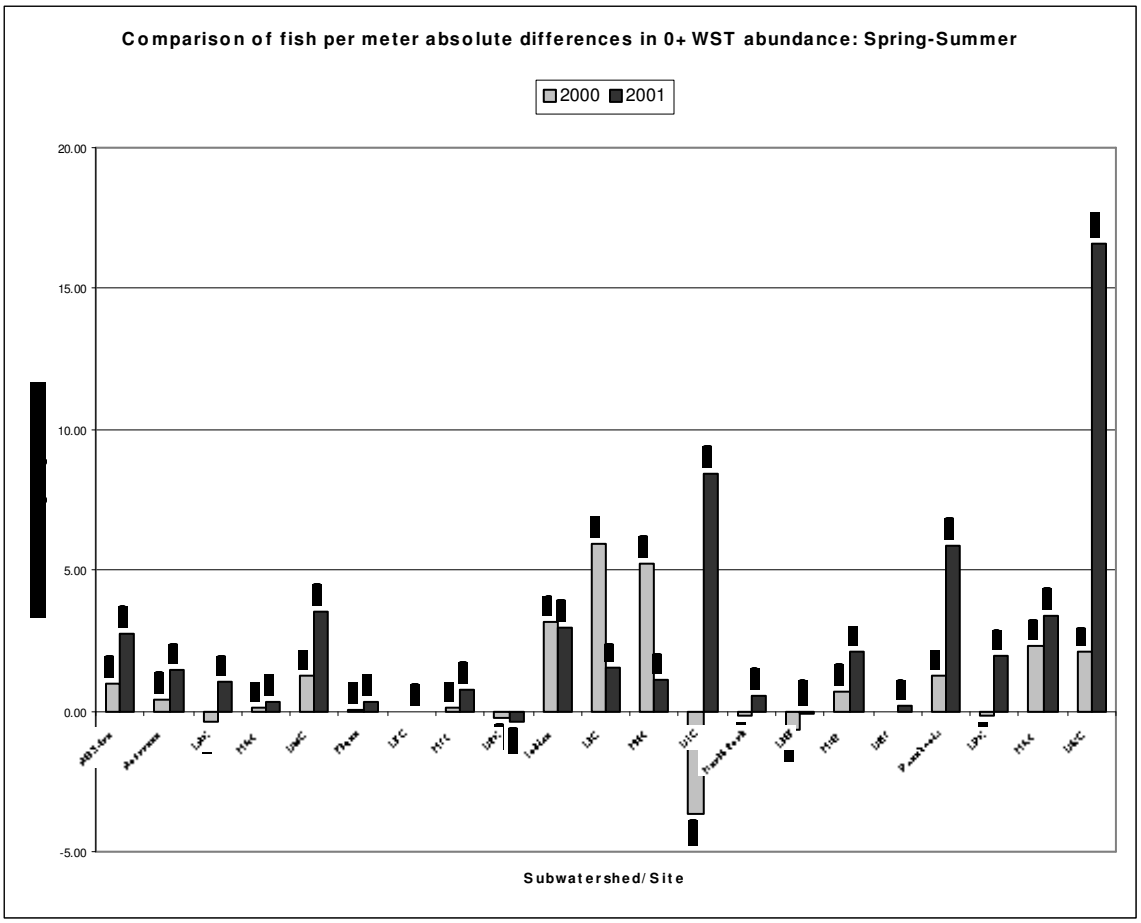


Figure 1-8. Differences between the spring and summer sampling periods by site and watershed in the number of 0+ steelhead on a per meter basis. Positive numbers indicate that there were more fish in the spring than the summer. Negative numbers indicate more fish in the summer with the increase being the result of immigration to the site.

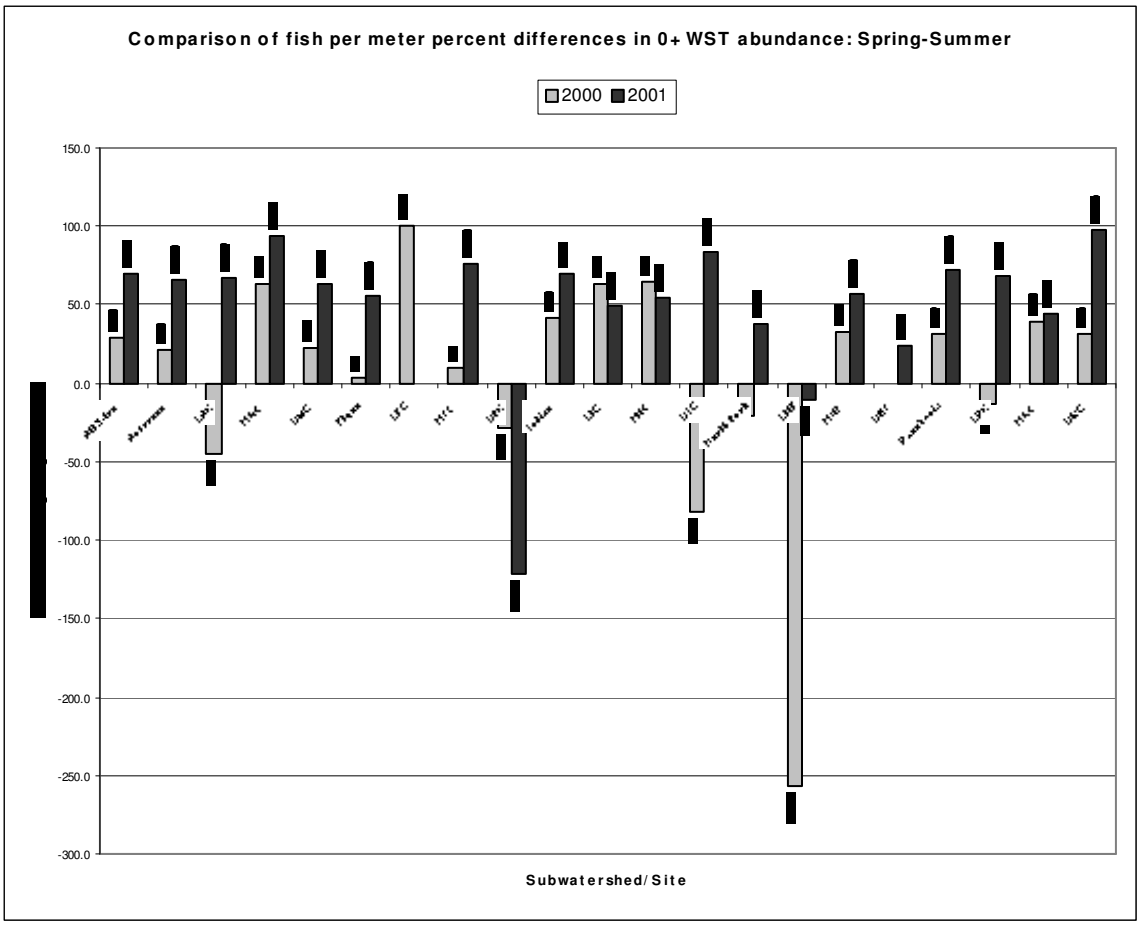


Figure 1-9. Percent differences between the spring and summer sampling periods by site and watershed in the number of 0+ steelhead on a per meter basis. The percentage is based on the number of fish present in the spring. Positive numbers indicate that there were more fish in the spring than the summer. Negative numbers indicate more fish in the summer with the increase being the result of immigration to the site.

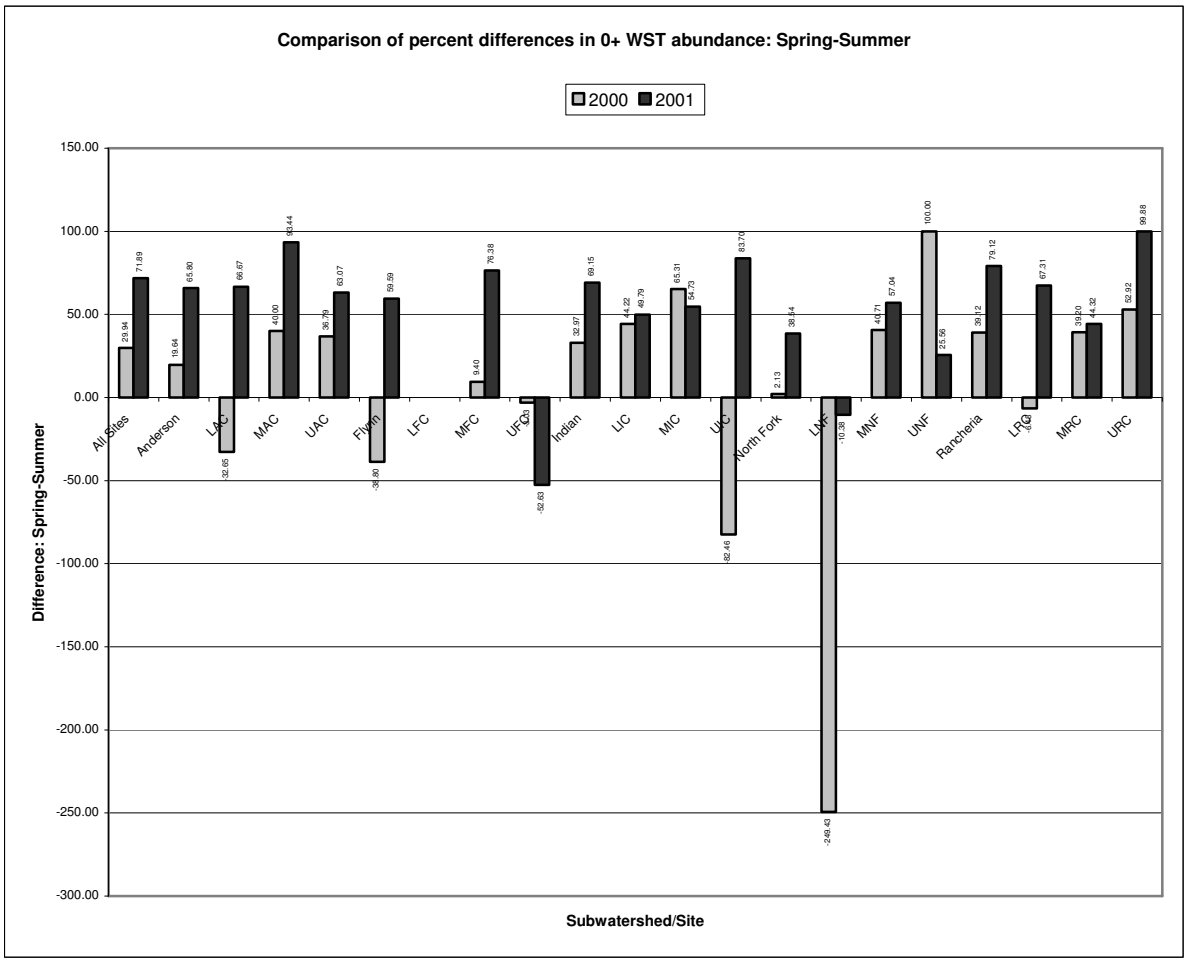


Figure 1-10. Percent differences between the spring and summer sampling periods by site and watershed in the number of 0+ steelhead. Percentage is based on the number of fish present in the spring. Positive numbers indicate that there were more fish in the spring than the summer. Negative numbers indicate more fish in the summer with the increase being the result of immigration to the site.

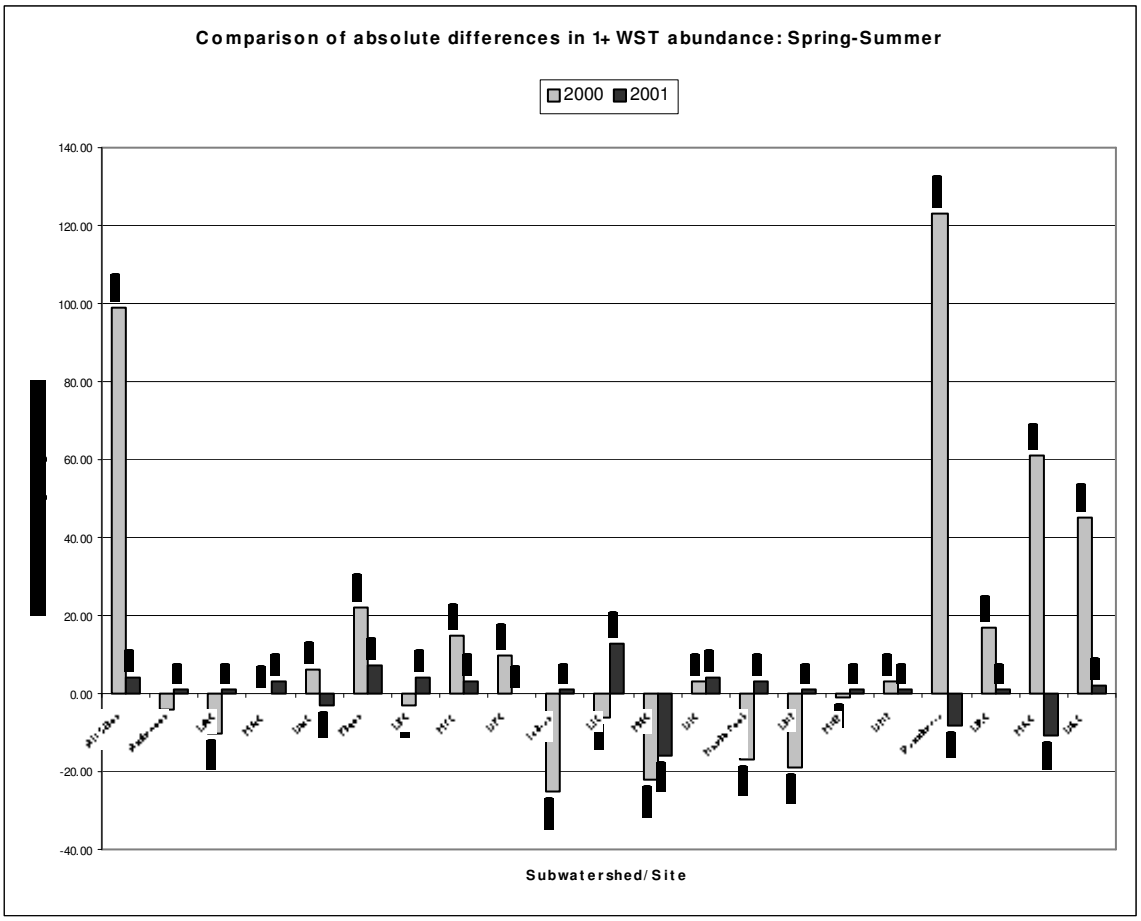


Figure 1-11. Differences between the spring and summer sampling periods by site and watershed in the number of 1+ steelhead. Positive numbers indicate that there were more fish in the spring than the summer. Negative numbers indicate more fish in the summer with the increase being the result of immigration to the site.

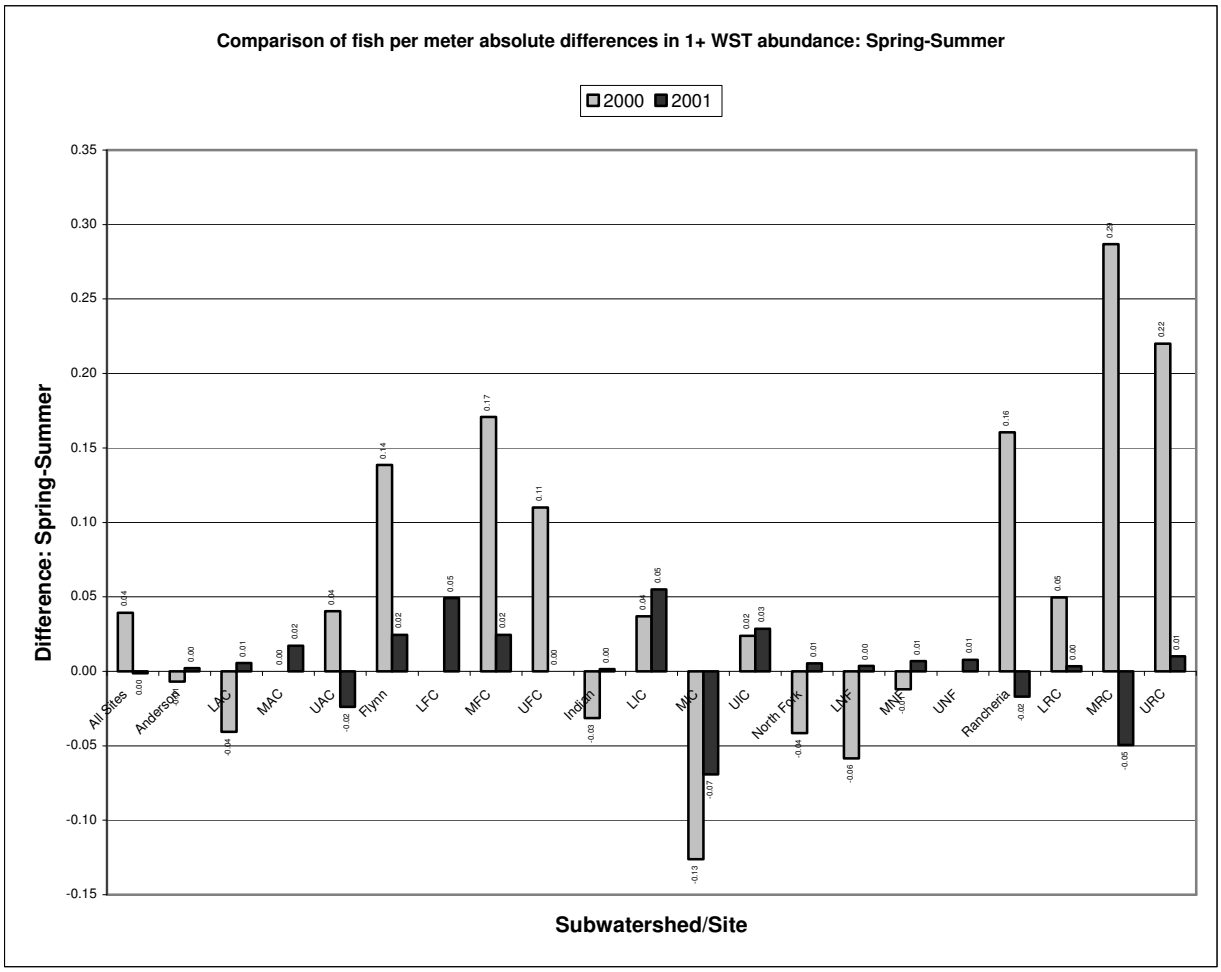


Figure 1-12. Differences between the spring and summer sampling periods by site and watershed in the number of 1+ steelhead on a per meter basis. Positive numbers indicate that there were more fish in the spring than the summer. Negative numbers indicate more fish in the summer with the increase being the result of immigration to the site.

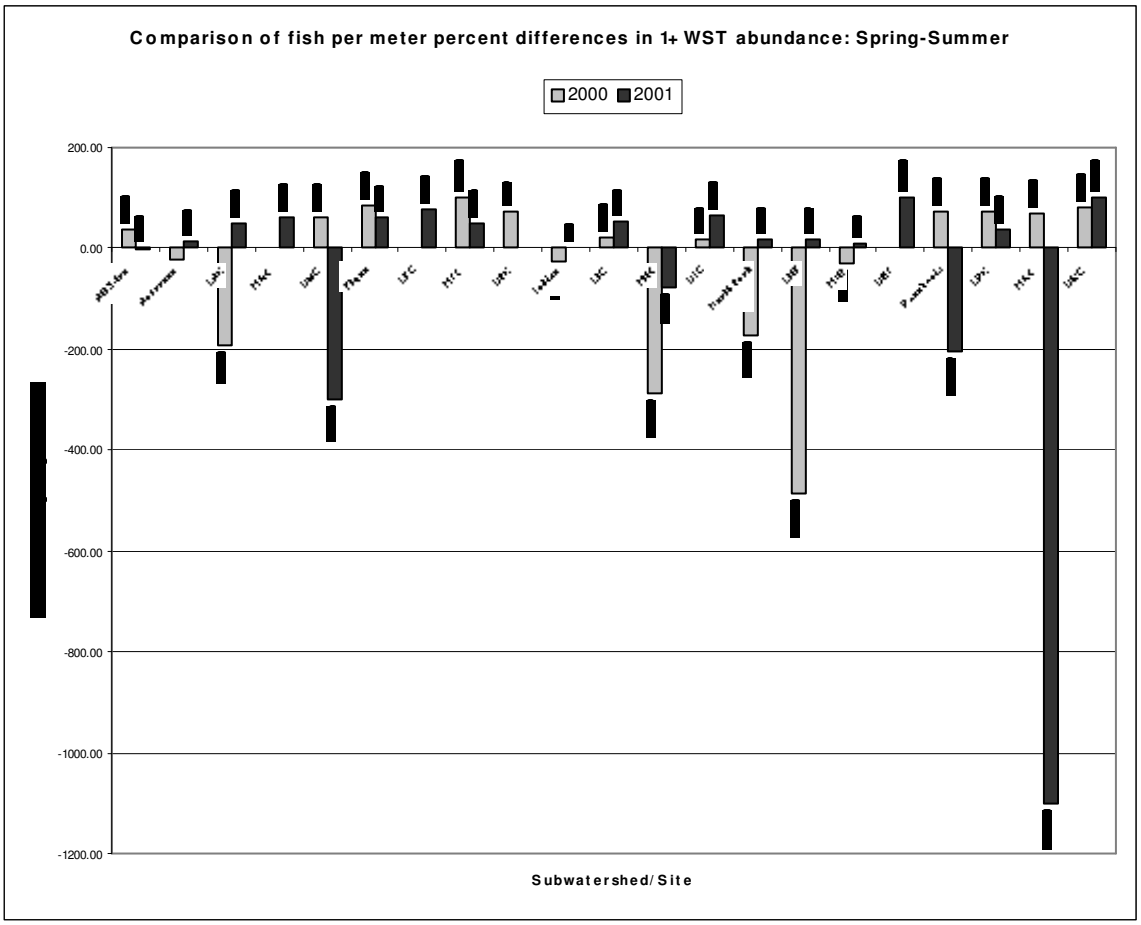


Figure 1-13. Percent differences between the spring and summer sampling periods by site and watershed in the number of 1+ steelhead on a per meter basis. The percentage is based on the number of fish present in the spring. Positive numbers indicate that there were more fish in the spring than the summer. Negative numbers indicate more fish in the summer with the increase being the result of immigration to the site.

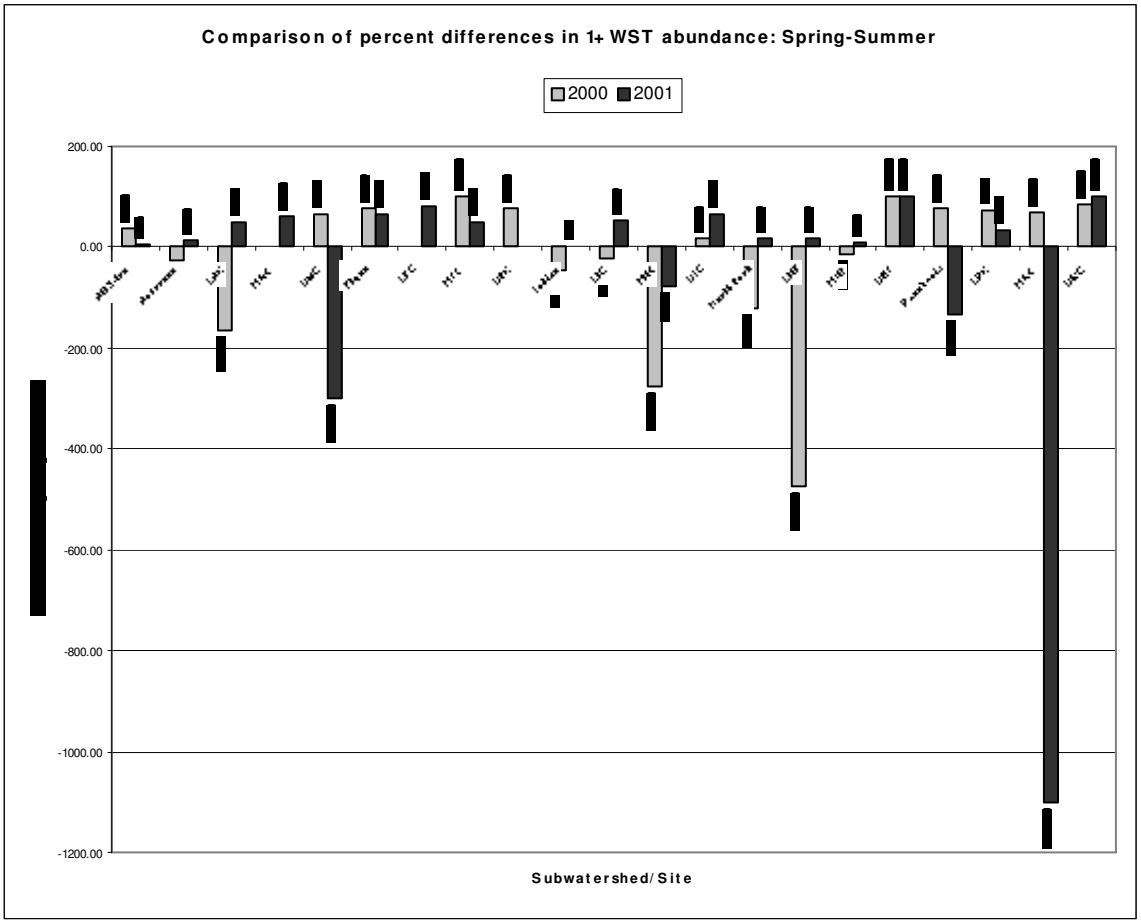


Figure 1-14. Percent differences between the spring and summer sampling periods by site and watershed in the number of 1+ steelhead. Percentage is based on the number of fish present in the spring. Positive numbers indicate that there were more fish in the spring than the summer. Negative numbers indicate more fish in the summer with the increase being the result of immigration to the site.

	Site														
Emergence	JSC	LAC	LFC	LIC	LNF	LRC	MAC	MFC	MIC	MNF	MRC	UAC	UIC	URC	Grand Total
3/3/2000														1	1
3/6/2000											1				1
3/7/2000							1								1
3/8/2000						1								1	2
3/9/2000									1					2	3
3/10/2000		1				1	1			1					4
3/11/2000									1						1
3/12/2000							1								1
3/14/2000		1											1		2
3/15/2000							1							1	2
3/16/2000		1								1					2
3/18/2000		2				1	1							1	5
3/19/2000		1												2	3
3/20/2000						1	1			3					5
3/21/2000						1			1	1			1	1	5
3/22/2000							1						1	1	3
3/23/2000						1				2			1		4
3/24/2000					1	1				1				1	4
3/25/2000					1					1	2		1	1	6
3/26/2000								1		1			1		3
3/27/2000	1				1	1				2				1	6
3/28/2000										1					1
3/29/2000				1	1					1			1		4
3/30/2000						1									1
3/31/2000	1													1	2
4/1/2000			1			1									2
4/2/2000					1	1								1	3
4/3/2000							1				1				2
4/4/2000	1		1			1							1		4
4/5/2000		1		1					1						3
4/6/2000			1			1			1	1			1	1	6
4/7/2000							1					1			2
4/8/2000	1				1		1				1	3	1		8
4/9/2000		1					5		1			2	1		10
4/10/2000				1	1			1	2			1			6
4/11/2000							1		1		2		2	1	7
4/12/2000									1	2			2	2	7
4/13/2000					1								1		2
4/14/2000	1	1	1		2	1					2	4	1	1	14
4/15/2000					1		1		1		1	1			5
4/16/2000		2	2				1			1	2	1	3	2	14
4/17/2000		4			1	1				3	4			2	15
4/18/2000		2							1		1		1		5
4/19/2000		1				1	1			2			2	1	8
4/20/2000		1	1					3	1						6
4/21/2000				1	3	1			1	2			1	1	10
4/22/2000	1				1				1			2	1	1	7
4/23/2000		1	1					1			2	3		1	9
4/24/2000								1	1			1		1	4
4/25/2000				1	2						1	1			5
4/26/2000							1	2							3
4/27/2000	1			1	1		1	2	1						7
4/28/2000	1						2	1		2				1	7
4/29/2000	1		1	1										1	4
4/30/2000		1			2				1						4
5/1/2000	1							2		1		1			5
5/2/2000				2				1	1	1			3		8
5/3/2000	1														1
5/4/2000				1		1		2			1	2			7
5/5/2000				2											2
5/6/2000	1	1		1			1	1				1			6
5/7/2000		1		5	2										8
5/8/2000					1					1	1	1			4
5/9/2000		3		2				1							6
5/10/2000				2								1			3
5/11/2000				2		1					1				4
5/13/2000				1											1
5/14/2000				2		1				1	1				5
5/15/2000						2									2

5/16/2000				1				1				1			3
5/17/2000					1					1					2
5/18/2000					1	1									2
5/19/2000				1		1					1				3
5/20/2000						1						1		1	3
5/21/2000												1			1
5/24/2000						1									1
5/25/2000								1							1
5/26/2000				1									1		2
5/27/2000						1									1
6/16/2000						1									1
Grand Total	12	26	9	30	26	28	24	21	19	33	25	29	29	32	343

Figure 1-15. Emergence dates for steelhead collected at the sample sites within the Navarro River watershed. Although collected at the site, it is not known that the fish emerged from the gravel at that site.

SUBWATERSHED	SITE	COUNT (N)	MEAN	ST. DEVIATION
Flynn		30	0.368	0.086
	LFC	9	0.397	0.092
	MFC	21	0.356	0.082
North Fork		71	0.483	0.116
	LNF	26	0.548	0.115
	MNF	33	0.435	0.098
	JSC	12	0.476	0.106
Indian		78	0.580	0.324
	LIC	30	0.774	0.386
	MIC	19	0.538	0.265
	UIC	29	0.407	0.132
Anderson		79	0.582	0.208
	LAC	26	0.607	0.135
	MAC	24	0.781	0.171
	UAC	29	0.396	0.010
Rancheria		84	0.454	0.146
	LRC	28	0.492	0.137
	MRC	25	0.541	0.148
	URC	31	0.350	0.075

Table 1-2. Average growth rates by site and subwatershed.

Genetic structure of steelhead populations

Determining genetic population structure is an essential element in the successful management and conservation of exploited, threatened, or endangered species. Genetic data can aid in clarifying relationships among populations, delineating conservation units, and estimating contributions to mixed-stock fisheries. Microsatellites have proven to be particularly useful in surveying genetic variation in a variety of salmonids (e.g. Angers et al. 1995, Banks et al. 2000, Garrant et al. 2000, Neraas and Spruell 2001). Traits such as high polymorphism, ready availability of published loci, and non-lethal sampling make them ideal for population genetic studies. Recent investigations into the genetic variation found in steelhead have been conducted to describe population structure (Parkinson 1984; Beecham 1999, 2000), define genetic differences between spawning runs (Nielsen and Fountain 1999), and estimate stock composition in mixed-stock fisheries (Beecham et al. 1999).

The purpose of this study was to utilize microsatellites to characterize the genetic variation within and among summer steelhead populations in tributaries of the Navarro River on the north coast of California. Information based on differences in microsatellite allele frequencies was used to assess genetic diversity within and among tributary populations, investigate genetic relationships between populations, and evaluate the possibility of using stock identification analysis based on individual assignment tests to determine stock composition in mixed-stock samples.

Materials and Methods

Sample Collection

Muscle and fin clip samples of juvenile summer steelhead were collected from six tributaries of the Navarro River between May and August of 2000 (Table 1-3, Fig. 1-16).

When possible, samples were collected from the upper, middle, and lower sections of each tributary. In the case of Flynn Creek and John Smith Creek, logistic constraints restricted sampling to a single middle location. In addition, a sample of ten smolts was taken from the estuary at the mouth of the Navarro River. Samples were collected with bag seines or by electrofishing, either frozen at -20° C or placed in DMSO storage buffer (20% DMSO, 0.25 M EDTA, NaCl to saturation, pH 7.8), and transported to the UC Davis Genomic Variation Laboratory.

Genetic Analyses

Whole genomic DNA was extracted from tissue samples using the Qiagen DNeasy™ Tissue Kit. DNA extracts served as templates for the polymerase chain reaction (PCR) used to amplify product for 9 microsatellite loci (Williamson et al. 2002) originally developed from Chinook salmon *Oncorhynchus tshawytscha* and 2 loci (Rexroad et al. 2002) originally developed from rainbow trout *O. mykiss* (Table 2). Amplifications of all microsatellite loci were carried out in 10 µl reactions containing 5.15 µl sterile dH_2O, 1.0 µl 10X PCR buffer, 0.40 µl 50 mM $MgCl_2$, 0.80 µl 2.5 mM dNTP mixture, 0.2 µl 1 µM forward primer labeled with one of three fluorescent dyes, 0.40 µl 10 µM unlabeled reverse primer, 0.05 µl *Taq* I polymerase (0.25 U total), and 2.0 µl DNA (approximately 50ng DNA total). Samples were first denatured for 5 min at 95° C, followed by 30-35 cycles of PCR amplification performed under the following conditions: 1 min at 95° C, 1 min at 52° C, and 2 min at 72° C, with a final extension of 5 min at 72° C.

PCR products were separated electrophoretically on a 5.5% polyacrylamide gel using the MJ Research BaseStation gel analysis system (MJ Research, Inc., San Francisco, CA). The Genescan 500 size standard (MJ Research, Inc.) labeled with a fourth fluorescent dye was run in each lane. Resultant gel images were analyzed using the Cartographer software from MJ Research.

Statistical Analyses

Genetic data were analyzed in two ways based on the original sampling scheme. Initially, all samples from a given tributary were analyzed as a single sample set (referred to as pooled samples) as is commonly done in population genetic analyses found in the published literature. This insures adequate numbers of fish are collected representing a large portion of the population, but assumes no within-stream heterogeneity among sample sites. In the second analysis, individual sampling locations were treated as independent samples (referred to as discrete samples) to investigate the amount of genetic variation found within as well as among tributaries and any effect the introduction of this second level of variation may have on the population structure results.

Data were analyzed using Genes in Populations 2.2 (designed by B. May and C. Krueger, written in C by W. Eng and E. Paul; program is available for download at http://animalscience.ucdavis.edu/extension/Gene.htm) and Arlequin 2.0 (Schneider et al. 2000). Allele frequencies (F), observed (H_o) and expected (H_e) heterozygosities, and inbreeding coefficients (F_{is}) were estimated for all populations at each locus, and nonparametric, exact–significance tests (exact θ significance tests and exact probability

tests) were used to evaluate sample genotype distributions for departures from Hardy-Weinberg expectations. Unbiased estimators of exact significance probabilities for the Hardy-Weinberg equilibrium tests were calculated using the Markov chain algorithm of Guo and Thompson (1992) with a Markov chain length of 100,000 steps. Gametic disequilibrium tests were also performed to test for linkage between loci. Patterns of genetic diversity and divergence within and between populations were evaluated using the analysis of molecular variance (AMOVA) of Excoffier et al. (1992), which generates F-statistics analogous to the θ values of Wier and Cockerham (1984). Significance of F-statistics was evaluated using exact F permutation procedures (Excoffier et al. 1992). Type I error was controlled for all multiple testing using the sequential Bonferroni method of Rice (1989).

Graphical representations of relationships between samples in the form of unweighted pair group method with arithmetic means (UPGMA) dendrograms were constructed using Phylip 3.5c (Felsenstein 1995). The original allele frequency matrix was resampled 1000 times by bootstrapping and Nei's (1972) genetic distance (D) between samples was estimated for each resulting matrix. A consensus UPGMA diagram was then generated with the original branch lengths and all bootstrap values above 50% were plotted on to the dendrogram to indicate stability of the nodes.

Assignment tests were performed on the pooled samples using the WHICHRUN software of Banks and Eichert (2000). A jackknife procedure was first used to determine the ability of the program to correctly assign randomly chosen individuals back to their

original tributaries based on the expected genotypic frequencies of each pooled sample. We then attempted to assign individual genotypes of smolts from the Navarro River estuary to a source tributary, with the confidence of each assignment based on the log of the odds ratio (LOD) score for the two most likely source samples. If the ratio of the most likely source to the second most likely source approaches one, there is ambiguity in the assignment of that particular individual. Individuals with a large ratio in comparison to all other ratios can be assigned to a single source with more confidence. For the two populations considered in the ratio, the chance of error is equal to the inverse of this ratio, so that assignments that have a log of the odds (LOD) ratio >2 will have a 1/100 chance of error or less ($P<0.01$, Banks and Eichert 2000)

Results

Allele Frequencies and Heterozygosities

Pooled samples. The number of alleles exhibited by the microsatellite markers ranged from eight for the OtsG243 locus to 33 for OtsG85 (Table 1-4). Allele sizes in base pairs (bp), allele frequencies (F), observed heterozygosities (H_o), expected heterozygosities (H_e), inbreeding coefficient (F_{is}), and the sample size (N) for each locus in the six pooled samples of Navarro River steelhead are given in Table 1-5. Observed heterozygosities varied widely among loci and samples, ranging from a low of 0.188 for Flynn Creek (OtsG243) to a high of 1.00 for 7 of the population/locus combinations (Table 1-6). Average H_o values for individual loci calculated across populations ranged from 0.447 for OtsG003 to 0.936 for OtsG83b. Average H_o values for individual populations calculated across loci ranged from a low of 0.783 for Indian Creek to a high of 0.888 for John Smith Creek. Conformity of allele frequency distributions for all populations and

loci to Hardy-Weinberg equilibrium was calculated based on a significance level of p = 0.0007 (Rice 1989). Significant deviations from Hardy-Weinberg expectations occurred in samples from Flynn Creek at OtsG253 and Indian Creek at OtsG43. Deviations were a result of heterozygote excess for Flynn Creek/OtsG253 and heterozygote deficiency for Indian Creek/OtsG43. There was no evidence of linkage disequilibrium between any pair of loci after correction for multiple tests (p = 0.001).

Discrete Samples. Allele sizes in base pairs (bp), allele frequencies (F), observed heterozygosities (H_o), expected heterozygosities (H_e), inbreeding coefficient (F_{is}), and the sample size (N) for each locus in the 13 discrete samples of Navarro River steelhead are given in Table 1-7. Observed heterozygosities reflected those of the pooled samples, ranging from a low of 0.188 for Flynn Creek (OtsG243) and Middle Rancheria Creek (OtsG3) to a high of 1.00 for 19 of the population/locus combinations (Table 1-7). Average H_o values for individual loci calculated across populations ranged from 0.438 for OtsG3 to 0.931 for OtsG83b (Table 1-8). Average H_o values for individual populations calculated across loci ranged from a low of 0.745 for Middle Indian Creek to a high of 0.888 for John Smith Creek. A single significant deviation from Hardy-Weinberg expectations due to an excess of heterozygotes occurred in the Flynn Creek sample at OtsG253 (as would be expected, since there was only a single Flynn Creek sample, and so the 'pooled' and 'discrete' samples would be identical). The significant departure from Hardy-Weinberg equilibrium seen in the pooled Indian Creek sample was not present in the discrete sample analysis.

Population Structure Analysis

Pooled samples. Results of the analysis of molecular variance (AMOVA) test on the combined loci data set for the six, pooled samples revealed a highly significant amount of differentiation among the various creeks ($p < 0.001$, Table 1-9), with 2.9% of the overall variance attributable to among population differences. Likewise, pairwise population F_{st} values (Table 1-10) revealed significant differences ($p < 0.003$) between all creeks, with the highest pairwise F_{st} values observed between Flynn Creek and all other tributaries.

Genetic relationships among samples were visualized by constructing a UPGMA dendrogram based on Nei's (1972) genetic distances (Fig. 1-17). Genetic relationships among samples were completely congruent with geographic proximity of the various creeks. The Flynn Creek sample fell out as the most genetically distinct branch, with a D of 0.391. The John Smith Creek sample followed as the next most genetically distant sample with a D of 0.229,while genetic distances between the remaining samples ranged from 0.057-0.128. Bootstrap values ranged from 83.0%-100%, with 100% support for the nodes separating Flynn Creek and John Smith Creek from the remaining samples.

Discrete samples. Results of the analysis of molecular variance (AMOVA) test on the combined loci data set for the 13 discrete samples revealed a highly significant amount of differentiation both within and among the various creeks ($P < 0.001$, Table 1-11). Both variation among creeks and variation among sampling sites within creeks accounted for approximately 2% of the overall genetic variance. Pairwise population F_{st} values for the combined data revealed significant differences ($p< 0.0006$) between most but not all

samples (Table 1-12). Upper Indian Creek did not differ significantly from Upper, Middle, and Lower Anderson; Middle Anderson Creek did not differ significantly from Middle Richardson Creek; and Lower Richardson Creek did not differ significantly from Upper Indian Creek, Upper, Middle, and Lower Anderson Creek, or Upper and Middle Richardson Creek.

Genetic relationships among samples were again visualized by constructing a UPGMA dendrogram of genetic distances (Figure 1-17). The Flynn Creek sample still fell out as the most genetically distinct branch (D =0.437) with 100% bootstrap support. Likewise, the John Smith Creek sample again followed as the next most genetically distant sample (D=0.277) with a 67.5% bootstrap value. The remaining genetic distances ranged between 0.057 and 0.128 and exhibited less congruence with geographic proximity of samples; a number of within-creek samples did not group together, and bootstrap values were uniformly low. Although the three Rancheria Creek samples did form a terminal clade, bootstrap support for the node separating them from the rest of the samples was only 10.2%.

Assignment Testing

Results of the jackknife test indicated a wide range of values for the proportion of individuals that were correctly assigned back to their original population (Table 1-13). The proportion of correct assignments was high for the two most genetically distinct populations (94% for Flynn Creek and 100% for John Smith Creek). Values for the four remaining samples were much lower, ranging from 52% for the Indian Creek Sample to 32% for Anderson Creek. With the jackknife results in mind, an attempt was made to

assign steelhead smolt collected in the Navarro River estuary back to their source population. Three individuals each were assigned to Flynn Creek and North Fork, and two each to John Smith Creek and Rancheria Creek (Table 1-14). LOD scores ranged from 1.22 to 2240.00, so that all but three of the assignments (Individual # 3, 8, and 10) had a 1/100 or less chance of being in error.

Discussion

Average observed heterozygosities for pooled (range = 0.783-0.888) and discrete (0.745-0.888) samples calculated across loci were similar to those seen in other population genetic studies utilizing microsatellites in steelhead trout from northern British Columbia (Beecham et al. 1999, Heath et al. 2002), southern British Columbia, Washington, and the Columbia River (Beecham et al. 2000), and the Middle Fork Eel River in California (Nielsen and Fountain 1999). AMOVA results and pairwise population F_{st} values for the pooled samples indicated significant differences in genetic variation among the six Navarro River tributaries, suggesting limited contemporary gene flow among tributaries in the Navarro watershed. AMOVA results for the discrete samples, however, also indicated significant differences at the within-creek level; this accounted for an amount of the overall variance equal to that explained by the among-creek level. In addition, pairwise population F_{st} values indicated significant differences between some within-creek sites, as well as non-significance between some sites from different creeks. Various explanations can be invoked to account for these results. It could reflect a real case of restricted gene flow between sampling sites, though this seems unlikely. It could be a consequence of having sampled related juveniles (Allendorf-Phelps effect; Waples

1998), although the lack of any widespread significant deviations from Hardy-Weinberg equilibrium argues against this cause. Finally, it could be a result of sampling error due to the small sample sizes used when the creeks were split into upper, middle, and lower stretches. These small sizes may have generated sample allele frequency distributions that did not accurately reflect those of the real populations, and resulted in apparent within-creek differences in genetic variation. In any case, failure to account for within-creek heterogeneity would have led to an inflated value for the percentage of variance ascribed to among–creek variation (2.92 vs. 1.89).

Genetic distances between Navarro river tributaries were comparable to those reported in the literature for other steelhead populations. Values for Nei's genetic distance D between discrete samples (range=0.057-0.437) were very similar to those reported by Heath et al. (2002) for three steelhead populations (range=0.109-0.523) sampled over multiple years in the Skeena River watershed of British Columbia based on seven microsatellite loci. These values were on average nearly an order of magnitude greater than the genetic distance between winter and summer run steelhead in the Middle Fork Eel River (Nielsen and Fountain 1999).

The relationships among Navarro River tributaries based on the analysis of pooled samples (Figure 1-16) were quite robust and in complete accord with geographic distances among tributaries. These relationships broke down to some extent based on the discrete sample analysis, however, indicating that larger within-creek sample sizes and multiple-year samples may be required to confirm the results presented here. An

example of the dangers of not properly sampling is given in Garant et al. (2000). These researchers found significant within-stream and inter-annual heterogeneity in microsatellite allele frequencies in Atlantic salmon taken from four tributaries of the Sainte-Marguerite River in Quebec, Canada. The genetic variance attributable to these factors was nearly three times more important than the genetic differentiation found among tributaries.

The ability to correctly assign individual genotypes back to their original populations was proportional to the genetic distances among the various tributaries. Proper assignment of individuals to Flynn and John Smith Creeks, the two most genetically distinct populations, approached 100%. In contrast, correct assignments to the other, less genetically distinct tributaries averaged below 50%. Assignments of the Navarro estuary smolts to source populations were accompanied with high LOD scores in many cases, indicating relatively high confidence in the accuracy of the assignments. These results should be viewed with caution, however, since accurate assignment is highly dependent on proper representation of all possible source populations. Nevertheless, the results presented here suggest that with proper sampling, it may be possible to estimate the contribution of Navarro River tributaries to the outgoing smolt population found in the river's estuary, as well as using mixed stock analyses (MSA) to estimate the contribution of Navarro River tributaries to existing steelhead fisheries.

Table 1-3. Samples of summer steelhead taken for genetic analysis from the Navarro River and its tributaries in the summer of 2000.

Location	Population Code	Collection Dates	Sample sizes (n)	Latitude	Longitude
Navarro R. Estuary	NE	10/09/00	10	-123.760	39.192
Flynn Cr.	FC	06/09/00	16	-123.598	39.185
John Smith Cr.	JC	05/25/00	13	-123.531	39.222
Middle North Fork	MNF	05/25/00	24	-123.560	39.173
Lower North Fork	LNF	05/25/00	17	-123.585	39.159
Upper Indian Cr.	UIC	06/06/00	19	-123.375	39.078
Middle Indian Cr.	MIC	05/24/00	19	-123.428	39.071
Lower Indian Cr.	LIC	05/24/00	20	-123.440	39.059
Upper Anderson Cr.	UAC	06/08/00	20	-123.315	38.990
Middle Anderson Cr.	MAC	05/26/00	24	-123.372	39.014
Lower Anderson Cr.	LAC	05/26/00	19	-123.433	39.054
Upper Rancheria Cr.	URC	06/08/00	25	-123.239	38.850
Middle Rancheria Cr.	MRC	05/26/00	19	-123.324	38.948
Lower Rancheria Cr.	LRC	06/07/00	18	-123.440	39.054

Table 1-4. Microsatellite loci (with number of alleles per locus) used in this study.

Loci	No. of Alleles	Reference
OTSG3	15	Williamson et al. (2001)
OTSG43	17	‘ ’
OTSG83b	22	‘ ’
OTSG85	33	‘ ’
OTSG243	8	‘ ’
OTSG249b	23	‘ ’
OTSG253	24	‘ ’
OTSG401	17	‘ ’
OTSG423	23	‘ ’
OMM1082	18	Rexroad et al. (2002)
OMM1087	16	‘ ’

Table 1-5a-k. Allele sizes (in bp), allele frequencies, observed heterozygosities (H_o), expected heterozygosities (H_s), inbreeding coefficient (F_{is}), and number of sample individuals (N) for 11 microsatellite loci in pooled steelhead samples from six Navarro River tributaries.

a.

			OtsG3			
Alleles	FC	JC	NF	IC	AC	RC
138	0.00	0.19	0.11	0.05	0.063	0.04
142	0.00	0.00	0.00	0.00	0.024	0.00
146	0.84	0.69	0.63	0.72	0.714	0.75
150	0.00	0.00	0.04	0.05	0.024	0.04
170	0.00	0.00	0.15	0.00	0.024	0.06
174	0.00	0.00	0.00	0.00	0.00	0.01
182	0.00	0.00	0.04	0.07	0.024	0.01
186	0.16	0.04	0.02	0.05	0.04	0.04
190	0.00	0.08	0.01	0.02	0.04	0.03
194	0.00	0.00	0.00	0.00	0.008	0.02
202	0.00	0.00	0.00	0.01	0.024	0.00
206	0.00	0.00	0.00	0.00	0.008	0.00
210	0.00	0.00	0.00	0.01	0.008	0.01
226	0.00	0.00	0.00	0.00	0.00	0.01
246	0.00	0.00	0.00	0.02	0.00	0.00
H_o	0.31	0.62	0.46	0.38	0.51	0.40
H_s	0.26	0.48	0.56	0.47	0.48	0.44
F_{is}	-0.18	-0.29	0.17	0.19	-0.06	0.07
N	16	13	41	50	63	57

b.

			OtsG43			
Alleles	FC	JC	NF	IC	AC	RC
144	0.00	0.00	0.00	0.00	0.00	0.01
148	0.26	0.00	0.27	0.14	0.18	0.21
152	0.19	0.42	0.23	0.31	0.40	0.51
156	0.03	0.00	0.08	0.17	0.06	0.04
160	0.00	0.00	0.01	0.07	0.06	0.02
164	0.19	0.15	0.04	0.05	0.02	0.02
168	0.26	0.15	0.10	0.02	0.06	0.04
172	0.06	0.08	0.17	0.00	0.01	0.02
176	0.00	0.00	0.00	0.00	0.03	0.00
180	0.00	0.00	0.00	0.01	0.06	0.01
184	0.00	0.15	0.05	0.01	0.01	0.00
188	0.00	0.00	0.01	0.02	0.02	0.00
192	0.00	0.00	0.01	0.12	0.06	0.11
196	0.00	0.04	0.01	0.07	0.00	0.01
200	0.00	0.00	0.00	0.00	0.01	0.00
212	0.00	0.00	0.00	0.00	0.01	0.00
216	0.00	0.00	0.00	0.00	0.01	0.01
Ho	0.93	0.77	0.92	0.67	0.82	0.70
Hs	0.79	0.74	0.82	0.827	0.79	0.68
Fis	-0.19	-0.04	-0.12	0.19	-0.05	-0.04
N	15	13	38	49	63	61

c.

	OtsG83b					
Alleles	FC	JC	NF	IC	AC	RC
93	0.00	0.00	0.01	0.01	0	0.01
97	0.00	0.08	0.17	0.15	0.19	0.15
101	0.00	0.00	0.00	0.00	0.00	0.00
105	0.00	0.00	0.00	0.03	0.02	0.00
109	0.00	0.00	0.01	0.00	0.02	0.01
113	0.00	0.00	0.02	0.00	0.09	0.09
117	0.00	0.00	0.04	0.03	0.07	0.01
121	0.00	0.04	0.07	0.08	0.05	0.08
125	0.22	0.00	0.06	0.05	0.08	0.06
129	0.10	0.00	0.13	0.12	0.11	0.07
133	0.00	0.23	0.05	0.03	0.02	0.13
137	0.00	0.00	0.04	0.08	0.04	0.04
141	0.19	0.08	0.02	0.03	0.09	0.05
145	0.00	0.08	0.05	0.07	0.09	0.03
149	0.31	0.12	0.12	0.14	0.02	0.08
153	0.00	0.04	0.06	0.06	0.03	0.04
157	0.16	0.00	0.06	0.10	0.06	0.15
161	0.03	0.23	0.06	0.01	0.02	0.01
165	0.00	0.00	0.00	0.01	0.00	0.00
173	0.00	0.08	0.00	0.00	0.00	0.00
193	0.00	0.04	0.01	0.01	0.00	0.00
205	0.00	0.00	0.00	0.00	0.01	0.00
Ho	0.94	1.00	0.93	0.98	0.81	0.96
Hs	0.78	0.85	0.91	0.90	0.91	0.90
Fis	-0.19	-0.17	-0.02	-0.08	0.11	-0.07
N	16	13	41	52	63	54

d.

			OtsG85			
Alleles	FC	JC	NF	IC	AC	RC
1127	0.00	0.00	0.00	0.019	0.02	0.00
131	0.38	0.00	0.01	0.00	0.00	0.00
135	0.00	0.04	0.07	0.03	0.04	0.06
139	0.00	0.19	0.21	0.29	0.19	0.08
143	0.00	0.00	0.01	0.00	0.00	0.02
147	0.00	0.00	0.00	0.01	0.00	0.01
151	0.00	0.00	0.00	0.01	0.02	0.08
155	0.03	0.00	0.00	0.01	0.00	0.02
159	0.03	0.08	0.00	0.03	0.06	0.02
163	0.22	0.19	0.08	0.02	0.02	0.06
167	0.00	0.00	0.07	0.03	0.04	0.07
171	0.00	0.00	0.04	0.04	0.04	0.07
175	0.00	0.08	0.07	0.08	0.09	0.11
179	0.00	0.00	0.02	0.02	0.03	0.06
183	0.00	0.00	0.01	0.01	0.02	0.02
187	0.00	0.00	0.00	0.06	0.06	0.02
191	0.00	0.08	0.01	0.01	0.05	0.02
195	0.00	0.00	0.05	0.01	0.02	0.00
199	0.09	0.04	0.08	0.03	0.02	0.04
203	0.00	0.19	0.05	0.12	0.05	0.02
207	0.00	0.00	0.02	0.08	0.00	0.00
211	0.00	0.04	0.02	0.01	0.02	0.02
215	0.00	0.04	0.05	0.04	0.06	0.02
219	0.06	0.00	0.04	0.03	0.07	0.07
223	0.00	0.00	0.00	0.00	0.04	0.04
227	0.03	0.00	0.00	0.00	0.00	0.00
231	0.00	0.00	0.00	0.01	0.00	0.00
235	0.00	0.00	0.01	0.00	0.00	0.00
239	0.00	0.00	0.00	0.01	0.00	0.01
243	0.16	0.04	0.01	0.01	0.02	0.00
247	0.00	0.00	0.02	0.00	0.02	0.00
251	0.00	0.00	0.00	0.00	0.02	0.06
295	0.00	0.00	0.01	0.00	0.00	0.00
Ho	0.94	1.00	0.93	0.85	0.86	0.88
Hs	0.77	0.86	0.91	0.88	0.92	0.94
Fis	-0.22	-0.16	-0.02	0.04	0.07	0.06
N	16	13	41	52	63	60

e.

OtsG243						
Alleles	FC	JC	NF	IC	AC	RC
106	0.00	0.00	0.00	0.00	0.00	0.02
110	0.88	0.39	0.57	0.50	0.46	0.40
112	0.00	0.31	0.02	0.18	0.06	0.08
114	0.03	0.12	0.30	0.19	0.33	0.33
118	0.03	0.12	0.10	0.10	0.12	0.13
122	0.00	0.08	0.00	0.03	0.02	0.03
126	0.03	0.00	0.00	0.00	0.00	0.00
130	0.03	0.00	0.00	0.00	0.00	0.00
Ho	0.19	0.77	0.71	0.69	0.71	0.65
Hs	0.23	0.72	0.57	0.67	0.66	0.70
Fis	0.19	-0.06	-0.24	-0.03	-0.08	0.08
N	16	13	41	52	63	60

f.

OtsG249b						
Alleles	FC	JC	NF	IC	AC	RC
131	0.00	0.00	0.00	0.01	0.00	0.02
139	0.22	0.04	0.26	0.12	0.11	0.08
143	0.00	0.19	0.04	0.04	0.02	0.08
147	0.00	0.12	0.04	0.01	0.04	0.02
151	0.00	0.08	0.00	0.04	0.10	0.04
155	0.00	0.04	0.10	0.16	0.18	0.11
159	0.25	0.19	0.16	0.12	0.09	0.09
163	0.25	0.15	0.18	0.04	0.05	0.03
167	0.09	0.12	0.13	0.07	0.10	0.09
171	0.00	0.00	0.02	0.06	0.04	0.08
175	0.03	0.04	0.06	0.07	0.04	0.08
179	0.00	0.00	0.00	0.07	0.06	0.04
183	0.00	0.00	0.01	0.01	0.02	0.06
187	0.00	0.00	0.00	0.01	0.02	0.03
189	0.00	0.00	0.00	0.00	0.01	0.00
191	0.00	0.04	0.00	0.01	0.05	0.06
195	0.00	0.00	0.00	0.07	0.02	0.02
199	0.00	0.00	0.00	0.07	0.02	0.02
203	0.16	0.00	0.00	0.01	0.02	0.02
207	0.00	0.00	0.00	0.00	0.02	0.00
211	0.00	0.00	0.00	0.00	0.00	0.02
215	0.00	0.00	0.00	0.00	0.02	0.00
219	0.00	0.00	0.00	0.01	0.00	0.00
Ho	1.00	1.00	0.83	0.78	0.87	0.88
Hs	0.79	0.86	0.84	0.91	0.91	0.93
Fis	-0.26	-0.16	0.01	0.14	0.04	0.05
N	16	13	41	50	63	60

			OtsG253			
Alleles	FC	JC	NF	IC	AC	RC
140	0.00	0.04	0.01	0.04	0.02	0.09
144	0.00	0.00	0.00	0.00	0.00	0.02
148	0.00	0.00	0.01	0.04	0.04	0.00
156	0.00	0.08	0.00	0.00	0.03	0.02
160	0.00	0.08	0.08	0.00	0.02	0.00
164	0.00	0.00	0.05	0.00	0.04	0.02
168	0.00	0.04	0.03	0.13	0.07	0.09
172	0.19	0.04	0.10	0.10	0.15	0.09
176	0.09	0.04	0.00	0.021	0.02	0.05
180	0.00	0.12	0.30	0.27	0.16	0.12
184	0.03	0.15	0.10	0.10	0.10	0.15
188	0.25	0.23	0.04	0.07	0.07	0.04
192	0.03	0.00	0.08	0.01	0.05	0.06
196	0.00	0.00	0.05	0.05	0.05	0.05
200	0.00	0.12	0.05	0.14	0.09	0.03
204	0.41	0.04	0.01	0.01	0.02	0.09
208	0.00	0.00	0.04	0.00	0.02	0.03
212	0.00	0.00	0.03	0.01	0.01	0.04
216	0.00	0.00	0.01	0.01	0.00	0.01
220	0.00	0.00	0.01	0.00	0.01	0.00
232	0.00	0.00	0.00	0.00	0.02	0.00
236	0.00	0.04	0.00	0.00	0.00	0.00
240	0.00	0.00	0.00	0.00	0.00	0.02
244	0.00	0.00	0.00	0.00	0.02	0.00
Ho	0.94	0.92	0.85	0.81	0.95	0.88
Hs	0.73	0.88	0.87	0.86	0.91	0.92
Fis	-0.29	-0.05	0.02	0.06	-0.04	0.04
N	16	13	39	47	62	52

h.

			OtsG401			
Alleles	FC	JC	NF	IC	AC	RC
168	0.50	0.00	0.11	0.07	0.04	0.10
176	0.00	0.04	0.01	0.00	0.02	0.05
180	0.09	0.04	0.06	0.02	0.01	0.01
184	0.00	0.15	0.05	0.01	0.02	0.02
188	0.03	0.12	0.12	0.08	0.10	0.05
192	0.00	0.00	0.11	0.06	0.08	0.09
196	0.00	0.12	0.02	0.04	0.04	0.05
200	0.00	0.00	0.08	0.09	0.13	0.07
204	0.22	0.08	0.17	0.16	0.22	0.10
208	0.12	0.15	0.06	0.11	0.15	0.12
212	0.03	0.08	0.11	0.08	0.13	0.22
216	0.00	0.00	0.02	0.10	0.02	0.02
220	0.00	0.08	0.02	0.02	0.02	0.07
224	0.00	0.00	0.04	0.01	0.02	0.01
228	0.00	0.00	0.00	0.08	0.00	0.00
232	0.00	0.12	0.00	0.08	0.01	0.01
236	0.00	0.00	0.00	0.01	0.00	0.00
Ho	0.62	0.85	0.85	0.88	0.84	0.83
Hs	0.68	0.88	0.90	0.91	0.88	0.89
Fis	0.08	0.04	0.05	0.03	0.04	0.06
N	16	13	41	51	62	60

i.

			OtsG423			
Alleles	FC	JC	NF	IC	AC	RC
79	0.00	0.04	0.00	0.00	0.00	0.02
83	0.00	0.08	0.00	0.00	0.00	0.00
87	0.0	0.04	0.04	0.04	0.10	0.14
91	0.00	0.00	0.01	0.01	0.06	0.04
95	0.00	0.23	0.10	0.12	0.09	0.12
99	0.00	0.00	0.01	0.01	0.02	0.09
103	0.28	0.08	0.15	0.07	0.05	0.02
107	0.06	0.04	0.08	0.05	0.02	0.06
111	0.06	0.12	0.11	0.12	0.25	0.08
115	0.41	0.00	0.06	0.05	0.03	0.04
119	0.00	0.00	0.06	0.05	0.06	0.05
123	0.00	0.19	0.09	0.15	0.07	0.08
127	0.16	0.00	0.05	0.01	0.02	0.02
131	0.00	0.04	0.00	0.01	0.00	0.02
135	0.00	0.04	0.05	0.01	0.00	0.02
139	0.00	0.00	0.02	0.01	0.02	0.03
143	0.00	0.04	0.00	0.05	0.01	0.01
147	0.00	0.00	0.04	0.09	0.02	0.03
151	0.00	0.00	0.05	0.08	0.10	0.05
155	0.00	0.04	0.05	0.02	0.01	0.02
159	0.00	0.00	0.02	0.02	0.03	0.00
163	0.00	0.00	0.00	0.05	0.03	0.02
167	0.00	0.04	0.00	0.00	0.01	0.01
Ho	0.88	1	0.98	0.94	0.90	0.88
Hs	0.72	0.87	0.92	0.92	0.89	0.92
Fis	-0.21	-0.15	-0.06	-0.03	-0.02	0.04
N	16	13	40	51	63	60

j.

			OMM1082			
Alleles	FC	JC	NF	IC	AC	RC
176	0.00	0.00	0.01	0.02	0.00	0.02
180	0.00	0.00	0.00	0.00	0.04	0.00
184	0.03	0.00	0.02	0.02	0.06	0.06
188	0.00	0.04	0.09	0.11	0.09	0.13
192	0.00	0.00	0.05	0.04	0.11	0.13
196	0.03	0.04	0.09	0.11	0.15	0.18
200	0.31	0.15	0.04	0.18	0.07	0.07
204	0.22	0.08	0.14	0.30	0.23	0.18
208	0.03	0.04	0.04	0.09	0.02	0.05
212	0.00	0.19	0.02	0.01	0.01	0.02
216	0.09	0.23	0.22	0.03	0.06	0.05
220	0.28	0.04	0.11	0.06	0.06	0.01
224	0.00	0.00	0.02	0.02	0.03	0.04
232	0.00	0.00	0.01	0.00	0.02	0.00
236	0.00	0.00	0.01	0.00	0.01	0.00
238	0.00	0.19	0.10	0.01	0.01	0.02
240	0.00	0.00	0.01	0.02	0.03	0.04
244	0.00	0.00	0.00	0.00	0.00	0.01
Ho	0.94	0.85	0.92	0.79	0.86	0.95
Hs	0.76	0.84	0.89	0.84	0.88	0.89
Fis	-0.23	-0.01	-0.05	0.06	0.03	-0.07
N	16	13	40	52	63	60

k.

	OMM1087					
Alleles	FC	JC	NF	IC	AC	RC
213	0.00	0.00	0.00	0.00	0.01	0.00
241	0.22	0.12	0.04	0.09	0.06	0.16
245	0.03	0.15	0.09	0.26	0.11	0.14
249	0.00	0.00	0.01	0.03	0.02	0.01
253	0.38	0.15	0.16	0.02	0.01	0.02
257	0.34	0.31	0.26	0.14	0.14	0.19
261	0.00	0.04	0.02	0.08	0.15	0.08
265	0.03	0.00	0.05	0.09	0.02	0.11
269	0.00	0.00	0.21	0.12	0.14	0.05
273	0.00	0.08	0.04	0.06	0.11	0.09
277	0.00	0.00	0.00	0.01	0.04	0.03
281	0.00	0.08	0.02	0.07	0.07	0.05
285	0.00	0.08	0.05	0.02	0.06	0.07
289	0.00	0.00	0.00	0.01	0.02	0.00
291	0.00	0.00	0.05	0.02	0.00	0.01
293	0.00	0.00	0.01	0.00	0.02	0.00
Ho	1.00	1.00	0.88	0.85	0.89	0.84
Hs	0.69	0.82	0.85	0.87	0.89	0.88
Fis	-0.45	-0.21	-0.04	0.02	0.01	0.05
N	16	13	41	52	62	61

Table 1-6. Observed Heterozygosities (H_o) for 11microsatellite loci and pooled steelhead samples from six Navarro River tributaries. H_o values that deviated significantly from Hardy-Weinberg expectations after correction for multiple tests (α=0.0003) are shown in boldface.

Loci	Populations						Locus Average
	FC	JC	NF	IC	AC	RC	
OtsG3	0.31	0.62	0.46	0.38	0.51	0.40	0.45
OtsG43	0.93	0.77	0.92	**0.67**	0.82	0.70	0.80
OtsG83b	0.94	1.00	0.93	0.98	0.81	0.96	0.94
OtsG85	0.94	1.00	0.93	0.85	0.86	0.88	0.91
OtsG243	0.19	0.77	0.71	0.69	0.71	0.65	0.62
OtsG249b	1.00	1.00	0.83	0.78	0.87	0.88	0.89
OtsG253	**0.94**	0.92	0.85	0.81	0.95	0.88	0.89
OtsG401	0.62	0.85	0.85	0.88	0.84	0.83	0.81
OtsG423	0.88	1.00	0.98	0.94	0.90	0.88	0.93
OMM1082	0.94	0.85	0.92	0.79	0.86	0.95	0.88
OMM1087	1.00	1.00	0.88	0.85	0.89	0.84	0.91
Population Average	0.79	0.89	0.84	0.78	0.82	0.81	----

Table 1-7. Allele sizes (in base pairs), allele frequencies (*F*), observed heterozygosities (H_o), expected heterozygosities (H_e), inbreeding coefficient (F_{is}), and number of sample individuals (*N*) for 11 microsatellite loci and 13 samples of Navarro River steelhead.

OtsG3

Alleles (in bp)	FC	JC	MNF	LNF	UIC	MIC	LIC	UAC	MAC	LAC	URC	MRC	LRC
138	0	0.19	0.12	0.088	0.05	0.04	0.06	0.08	0.04	0.08	0.02	0.06	0.03
142	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.05	0.00	0.00	0.00
146	0.84	0.69	0.56	0.735	0.63	0.85	0.72	0.8	0.67	0.68	0.70	0.88	0.69
150	0.00	0.00	0.00	0.088	0.13	0.00	0.00	0.00	0.02	0.05	0.09	0.00	0.03
170	0.00	0.00	0.21	0.059	0.00	0.00	0.00	0.00	0.06	0.00	0.09	0.03	0.06
174	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00
182	0.00	0.00	0.06	0.00	0.03	0.04	0.14	0.00	0.06	0.00	0.00	0.00	0.03
186	0.16	0.04	0.04	0.00	0.10	0.04	0.00	0.02	0.02	0.08	0.04	0.00	0.06
190	0.00	0.08	0.00	0.029	0.00	0.00	0.06	0.05	0.04	0.03	0.04	0.00	0.03
194	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.02	0.00	0.03
202	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00
206	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00
210	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.03	0.00	0.00	0.03
226	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03
246	0.00	0.00	0.00	0.00	0.03	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ho	0.31	0.62	0.54	0.35	0.47	0.23	0.39	0.35	0.62	0.53	0.52	0.19	0.44
He	0.26	0.48	0.62	0.44	0.57	0.28	0.45	0.35	0.54	0.51	0.50	0.23	0.50
Fis	-0.19	-0.29	0.12	0.20	0.17	0.17	0.14	0.00	-0.16	-0.03	-0.05	0.18	0.12
N	16	13	24	17	19	13	18	20	24	19	23	16	18

OtsG43

Alleles (in bp)	FC	JC	MNF	LNF	UIC	MIC	LIC	UAC	MAC	LAC	URC	MRC	LRC
144	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
148	0.26	0.00	0.30	0.24	0.19	0.09	0.12	0.15	0.27	0.10	0.24	0.19	0.19
152	0.19	0.42	0.30	0.15	0.33	0.36	0.25	0.42	0.40	0.37	0.58	0.42	0.50
156	0.03	0.00	0.07	0.09	0.11	0.36	0.12	0.08	0.04	0.05	0.04	0.028	0.06
160	0.00	0.00	0.00	0.03	0.08	0.18	0.00	0.02	0.04	0.13	0.02	0.03	0.00
164	0.19	0.15	0.02	0.06	0.06	0.00	0.08	0.05	0.00	0.03	0.02	0.00	0.06
168	0.26	0.15	0.05	0.18	0.06	0.00	0.00	0.02	0.02	0.16	0.00	0.11	0.03
172	0.07	0.077	0.19	0.15	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.028	0.03
176	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.05	0.00	0.00	0.00

180	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.08	0.04	0.08	0.00	0.00	0.03
184	0.00	0.15	0.05	0.06	0.00	0.00	0.02	0.00	0.00	0.03	0.00	0.00	0.00
188	0.00	0.00	0.00	0.03	0.00	0.00	0.05	0.02	0.02	0.00	0.00	0.00	0.00
192	0.00	0.00	0.02	0.00	0.14	0.00	0.18	0.10	0.08	0.00	0.06	0.3	0.08
196	0.00	0.04	0.00	0.03	0.00	0.00	0.18	0.00	0.00	0.00	0.00	0.00	0.03
200	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00
212	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00
216	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.02	0.00	0.00
Ho	0.93	0.77	0.91	0.94	0.61	0.36	0.90	0.80	0.75	0.95	0.72	0.67	0.72
He	0.79	0.74	0.77	0.85	0.81	0.69	0.84	0.77	0.75	0.80	0.60	0.74	0.70
Fis	-0.19	-0.04	-0.17	-0.10	0.24	0.48	-0.08	-0.04	0.01	-0.19	-0.20	0.09	-0.04
N	15	13	21	17	18	11	20	20	24	19	25	18	18

OtsG83b

Alleles (in bp)	FC	JC	MNF	LNF	UIC	MIC	LIC	UAC	MAC	LAC	URC	MRC	LRC
93	0.00	0.00	0.02	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.04
97	0.00	0.08	0.25	0.06	0.16	0.23	0.10	0.10	0.19	0.29	0.14	0.23	0.07
101	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
105	0.00	0.00	0.00	0.00	0.03	0.04	0.03	0.03	0.02	0.00	0.00	0.00	0.00
109	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.04
113	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.03	0.13	0.11	0.06	0.07	0.18
117	0.00	0.00	0.04	0.03	0.03	0.00	0.05	0.05	0.10	0.05	0.02	0.00	0.00
121	0.00	0.04	0.08	0.06	0.03	0.04	0.15	0.03	0.08	0.03	0.12	0.07	0.04
125	0.22	0.00	0.04	0.09	0.00	0.04	0.10	0.05	0.08	0.11	0.06	0.03	0.07
129	0.09	0.00	0.13	0.15	0.24	0.08	0.05	0.20	0.08	0.05	0.10	0.00	0.11
133	0.00	0.23	0.04	0.06	0.03	0.00	0.05	0.05	0.02	0.00	0.14	0.13	0.11
137	0.00	0.00	0.04	0.03	0.03	0.19	0.05	0.08	0.02	0.03	0.06	0.00	0.04
141	0.19	0.08	0.00	0.06	0.03	0.08	0.00	0.20	0.00	0.08	0.06	0.07	0.00
145	0.00	0.08	0.02	0.09	0.11	0.04	0.05	0.05	0.13	0.08	0.04	0.00	0.04
149	0.31	0.12	0.15	0.09	0.11	0.04	0.25	0.00	0.04	0.00	0.06	0.07	0.14
153	0.00	0.04	0.06	0.06	0.05	0.12	0.03	0.03	0.02	0.05	0.00	0.10	0.04
157	0.16	0.00	0.04	0.09	0.13	0.12	0.05	0.05	0.06	0.08	0.14	0.20	0.11
161	0.03	0.23	0.04	0.09	0.03	0.00	0.00	0.03	0.02	0.03	0.00	0.03	0.00
165	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00
173	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
193	0.00	0.04	0.00	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
205	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00
Ho	0.94	1.00	0.88	1.00	1.00	1.00	0.95	0.95	0.71	0.79	0.96	0.93	1.00
He	0.79	0.85	0.88	0.92	0.87	0.86	0.88	0.89	0.89	0.86	0.90	0.86	0.90
Fis	-0.19	-0.17	0.00	-0.09	0.15	0.16	-0.08	-0.07	0.21	0.09	-0.07	-0.09	-0.12

N	16	13	24	17	19	13	20	20	24	19	25	15	14

OtsG85

Alleles (in bp)	FC	JC	MNF	LNF	UIC	MIC	LIC	UAC	MAC	LAC	URC	MRC	LRC
1127	0.00	0.00	0.00	0.00	0.03	0.04	0.00	0.00	0.02	0.03	0.00	0.00	0.00
131	0.38	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
135	0.00	0.04	0.10	0.03	0.03	0.00	0.05	0.00	0.02	0.11	0.02	0.12	0.06
139	0.00	0.19	0.25	0.15	0.21	0.35	0.33	0.25	0.17	0.16	0.06	0.03	0.17
143	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00
147	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.03
151	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.02	0.03	0.10	0.12	0.03
155	0.03	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.03
159	0.03	0.08	0.00	0.00	0.05	0.04	0.00	0.10	0.02	0.08	0.00	0.06	0.03
163	0.22	0.19	0.04	0.15	0.03	0.04	0.00	0.03	0.02	0.00	0.06	0.06	0.06
167	0.00	0.00	0.10	0.03	0.03	0.00	0.05	0.05	0.06	0.00	0.08	0.03	0.08
171	0.00	0.00	0.04	0.03	0.05	0.04	0.03	0.05	0.02	0.05	0.08	0.06	0.06
175	0.00	0.08	0.08	0.06	0.00	0.04	0.18	0.08	0.15	0.03	0.10	0.12	0.11
179	0.00	0.00	0.02	0.03	0.05	0.00	0.00	0.03	0.04	0.03	0.10	0.00	0.06
183	0.00	0.00	0.00	0.03	0.00	0.04	0.00	0.00	0.06	0.00	0.02	0.06	0.00
187	0.00	0.00	0.00	0.00	0.13	0.00	0.03	0.10	0.06	0.00	0.04	0.00	0.00
191	0.00	0.08	0.00	0.03	0.00	0.04	0.00	0.08	0.04	0.03	0.02	0.03	0.03
195	0.00	0.00	0.02	0.09	0.03	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00
199	0.09	0.04	0.06	0.12	0.03	0.04	0.03	0.05	0.00	0.03	0.04	0.03	0.06
203	0.00	0.19	0.08	0.00	0.18	0.04	0.13	0.00	0.08	0.05	0.04	0.03	0.00
207	0.00	0.00	0.02	0.03	0.05	0.23	0.00	0.00	0.00	0.00	0.00	0.00	0.00
211	0.00	0.04	0.00	0.06	0.03	0.00	0.00	0.03	0.02	0.03	0.00	0.00	0.08
215	0.00	0.04	0.04	0.06	0.03	0.04	0.05	0.00	0.08	0.08	0.04	0.00	0.00
219	0.06	0.00	0.02	0.06	0.00	0.00	0.08	0.13	0.04	0.05	0.08	0.03	0.08
223	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.11	0.06	0.03	0.03
227	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
231	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00
235	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
239	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00
243	0.16	0.04	0.02	0.00	0.00	0.00	0.03	0.05	0.00	0.03	0.00	0.00	0.00
247	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00
251	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.02	0.15	0.03
295	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ho	0.94	1.00	0.92	0.94	0.84	0.85	0.85	0.70	0.92	0.95	0.92	0.82	0.89
He	0.77	0.86	0.89	0.91	0.89	0.81	0.83	0.88	0.92	0.92	0.93	0.92	0.92

Fis	-0.22	-0.16	-0.03	-0.03	0.05	-0.04	-0.02	0.20	0.00	-0.03	0.01	0.10	0.03
N	16	13	24	17	19	13	20	20	24	19	25	17	18

OtsG243

Alleles (in bp)	FC	JC	MNF	LNF	UIC	MIC	LIC	UAC	MAC	LAC	URC	MRC	LRC
106	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.00
110	0.88	0.39	0.50	0.68	0.42	0.54	0.55	0.53	0.52	0.32	0.40	0.49	0.31
112	0.00	0.31	0.00	0.06	0.16	0.23	0.18	0.03	0.02	0.16	0.00	0.14	0.11
114	0.03	0.12	0.40	0.18	0.21	0.15	0.20	0.20	0.44	0.34	0.40	0.23	0.34
118	0.03	0.12	0.10	0.09	0.21	0.08	0.00	0.23	0.02	0.13	0.16	0.06	0.17
122	0.00	0.08	0.00	0.00	0.00	0.00	0.08	0.03	0.00	0.05	0.04	0.00	0.06
126	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
130	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ho	0.19	0.77	0.83	0.53	0.79	0.69	0.60	0.75	0.54	0.90	0.60	0.71	0.71
He	0.23	0.73	0.58	0.50	0.71	0.63	0.62	0.63	0.54	0.74	0.65	0.68	0.74
Fis	0.19	-0.06	0.43	-0.06	0.11	0.10	0.03	-0.19	-0.01	-0.21	0.08	-0.04	0.04
N	16	13	24	17	19	13	20	20	24	19	25	17	17

OtsG249b

Alleles (in bp)	FC	JC	MNF	LNF	UIC	MIC	LIC	UAC	MAC	LAC	URC	MRC	LRC
131	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.04	0.00	0.00
139	0.22	0.04	0.22	0.21	0.11	0.17	0.1	0.1	0.15	0.08	0.10	0.12	0.03
143	0.00	0.19	0.02	0.06	0.03	0.00	0.08	0.00	0.06	0.00	0.02	0.15	0.11
147	0.00	0.25	0.02	0.06	0.00	0.00	0.02	0.05	0.06	0.00	0.02	0.06	0.00
151	0.00	0.08	0.00	0.00	0.00	0.04	0.08	0.12	0.17	0.00	0.02	0.06	0.06
155	0.00	0.04	0.08	0.12	0.19	0.08	0.18	0.25	0.08	0.21	0.12	0.09	0.11
159	0.25	0.19	0.17	0.15	0.08	0.29	0.05	0.02	0.12	0.10	0.08	0.06	0.14
163	0.25	0.15	0.19	0.18	0.08	0.04	0.00	0.02	0.10	0.00	0.00	0.03	0.08
167	0.09	0.12	0.12	0.15	0.11	0.00	0.08	0.12	0.08	0.08	0.16	0.03	0.06
171	0.00	0.00	0.02	0.03	0.03	0.12	0.05	0.05	0.04	0.03	0.04	0.18	0.03
175	0.03	0.04	0.06	0.06	0.19	0.00	0.00	0.02	0.04	0.05	0.10	0.15	0.00
179	0.00	0.00	0.00	0.00	0.06	0.00	0.12	0.02	0.00	0.16	0.08	0.00	0.03
183	0.00	0.00	0.02	0.00	0.00	0.00	0.02	0.05	0.00	0.03	0.08	0.03	0.06
187	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.02	0.00	0.04	0.00	0.06
189	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00
191	0.00	0.04	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.16	0.06	0.03	0.08
195	0.00	0.00	0.00	0.00	0.06	0.00	0.12	0.02	0.02	0.00	0.02	0.00	0.03
199	0.00	0.00	0.00	0.00	0.00	0.25	0.02	0.02	0.00	0.05	0.02	0.00	0.06
203	0.16	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.02	0.03	0.00	0.03	0.03

207	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00
211	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06
215	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.03	0.00	0.00	0.00
219	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ho	1.00	1.00	0.79	0.88	0.72	0.75	0.85	0.8	0.96	0.84	0.84	0.82	1.00
He	0.79	0.86	0.82	0.86	0.88	0.80	0.90	0.88	0.90	0.87	0.91	0.89	0.92
Fis	-0.26	-0.16	0.04	-0.03	0.18	0.06	0.06	0.09	-0.07	0.04	0.08	0.07	-0.09
N	16	13	24	17	18	12	20	20	24	19	25	17	18

OtsG253

Alleles (in bp)	FC	JC	MNF	LNF	UIC	MIC	LIC	UAC	MAC	LAC	URC	MRC	LRC
140	0.00	0.04	0.02	0.00	0.00	0.12	0.03	0.00	0.04	0.00	0.11	0.04	0.10
144	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00
148	0.00	0.00	0.00	0.03	0.07	0.08	0.00	0.00	0.10	0.00	0.00	0.00	0.00
156	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.03	0.06	0.00	0.02	0.04	0.00
160	0.00	0.08	0.04	0.13	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00
164	0.00	0.00	0.02	0.09	0.00	0.00	0.00	0.08	0.00	0.05	0.02	0.04	0.00
168	0.00	0.04	0.04	0.00	0.14	0.27	0.03	0.03	0.10	0.08	0.13	0.07	0.03
172	0.19	0.04	0.13	0.06	0.11	0.08	0.10	0.18	0.06	0.24	0.15	0.04	0.03
176	0.09	0.04	0.00	0.00	0.07	0.00	0.00	0.03	0.00	0.03	0.04	0.00	0.10
180	0.00	0.12	0.30	0.28	0.11	0.08	0.50	0.13	0.17	0.18	0.04	0.21	0.17
184	0.03	0.15	0.09	0.13	0.07	0.08	0.13	0.13	0.10	0.05	0.13	0.25	0.10
188	0.25	0.23	0.04	0.03	0.07	0.19	0.00	0.00	0.06	0.16	0.04	0.04	0.03
192	0.03	0.00	0.11	0.03	0.04	0.00	0.00	0.08	0.04	0.03	0.04	0.00	0.13
196	0.00	0.00	0.07	0.03	0.18	0.00	0.00	0.16	0.00	0.00	0.07	0.00	0.07

OtsG253 (continued)

Alleles (in bp)	FC	JC	MNF	LNF	UIC	MIC	LIC	UAC	MAC	LAC	URC	MRC	LRC
200	0.00	0.12	0.02	0.09	0.11	0.12	0.18	0.11	0.10	0.05	0.04	0.04	0.00
204	0.41	0.04	0.00	0.03	0.00	0.00	0.03	0.00	0.04	0.03	0.11	0.07	0.07
208	0.00	0.00	0.04	0.03	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.04	0.07
212	0.00	0.00	0.02	0.03	0.00	0.00	0.03	0.00	0.00	0.03	0.02	0.04	0.07
216	0.00	0.00	0.02	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00
220	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00
232	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.03	0.00	0.00	0.00
236	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
240	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.03
244	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00
Ho	0.94	0.92	0.87	0.81	0.79	0.77	0.85	0.95	1.00	0.90	0.91	0.79	0.93
He	0.73	0.88	0.86	0.86	0.89	0.84	0.69	0.88	0.91	0.86	0.90	0.87	0.90
Fis	-	-	-	0.06	0.12	0.09	-0.23	-0.08	-0.10	-0.04	-0.01	0.09	-0.03

	0.29	0.05	0.02										
N	16	13	23	16	14	13	20	19	24	19	23	14	15

OtsG401

Alleles (in bp)	FC	JC	MNF	LNF	UIC	MIC	LIC	UAC	MAC	LAC	URC	MRC	LRC
168	0.50	0.00	0.08	0.15	0.05	0.08	0.08	0.05	0.00	0.08	0.14	0.06	0.08
176	0.00	0.04	0.02	0.00	0.00	0.00	0.00	0.03	0.02	0.03	0.06	0.08	0.00
180	0.09	0.04	0.10	0.00	0.00	0.08	0.00	0.00	0.02	0.00	0.02	0.00	0.00
184	0.00	0.15	0.04	0.06	0.03	0.00	0.00	0.00	0.02	0.05	0.02	0.06	0.00
188	0.03	0.12	0.21	0.00	0.11	0.08	0.05	0.08	0.10	0.11	0.06	0.06	0.03
192	0.00	0.00	0.08	0.15	0.11	0.04	0.03	0.08	0.10	0.05	0.00	0.19	0.11
196	0.00	0.15	0.00	0.06	0.08	0.00	0.03	0.08	0.02	0.03	0.06	0.00	0.08
200	0.00	0.00	0.06	0.12	0.05	0.15	0.08	0.18	0.15	0.05	0.04	0.14	0.06
204	0.22	0.08	0.17	0.18	0.05	0.23	0.23	0.21	0.21	0.24	0.08	0.06	0.17
208	0.13	0.15	0.08	0.03	0.13	0.08	0.10	0.08	0.21	0.16	0.10	0.19	0.06
212	0.03	0.08	0.06	0.18	0.18	0.00	0.03	0.13	0.10	0.16	0.25	0.11	0.31
216	0.00	0.00	0.04	0.00	0.13	0.04	0.10	0.03	0.02	0.00	0.02	0.03	0.03
220	0.00	0.08	0.04	0.00	0.05	0.00	0.00	0.00	0.02	0.03	0.10	0.03	0.08
224	0.00	0.00	0.00	0.09	0.00	0.00	0.03	0.05	0.00	0.00	0.02	0.00	0.00
228	0.00	0.00	0.00	0.00	0.00	0.19	0.08	0.00	0.00	0.00	0.00	0.00	0.00
232	0.00	0.12	0.00	0.00	0.00	0.04	0.18	0.00	0.00	0.03	0.02	0.00	0.00
236	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ho	0.63	0.85	0.88	0.82	0.90	0.85	0.90	0.79	0.79	0.95	0.88	0.83	0.78
He	0.68	0.88	0.88	0.87	0.89	0.86	0.87	0.87	0.86	0.87	0.88	0.87	0.84
Fis	0.08	0.04	0.01	0.05	-0.01	0.01	-0.03	0.10	0.08	-0.09	0.00	0.04	0.07
N	16	13	24	17	19	13	19	19	24	19	24	18	18

OtsG423

Alleles (in bp)	FC	JC	MNF	LNF	UIC	MIC	LIC	UAC	MAC	LAC	URC	MRC	LRC
79	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.03	0.00
83	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
87	0.03	0.04	0.04	0.03	0.05	0.00	0.05	0.15	0.06	0.08	0.06	0.21	0.19
91	0.00	0.00	0.02	0.00	0.00	0.04	0.00	0.00	0.02	0.16	0.02	0.06	0.06
95	0.00	0.23	0.07	0.15	0.18	0.08	0.08	0.10	0.10	0.05	0.22	0.06	0.06
99	0.00	0.00	0.02	0.00	0.00	0.04	0.00	0.05	0.00	0.03	0.04	0.15	0.11
103	0.28	0.08	0.20	0.09	0.13	0.00	0.05	0.05	0.06	0.03	0.04	0.03	0.00
107	0.06	0.04	0.00	0.18	0.00	0.21	0.00	0.00	0.04	0.03	0.12	0.00	0.03
111	0.06	0.12	0.17	0.03	0.16	0.21	0.03	0.33	0.25	0.18	0.06	0.03	0.17

115	0.41	0.00	0.07	0.06	0.03	0.08	0.05	0.03	0.04	0.03	0.02	0.00	0.11
119	0.00	0.00	0.11	0.00	0.05	0.08	0.03	0.13	0.06	0.00	0.04	0.09	0.03
123	0.00	0.19	0.04	0.15	0.05	0.13	0.25	0.03	0.10	0.08	0.14	0.03	0.06
127	0.16	0.00	0.07	0.03	0.03	0.00	0.00	0.03	0.02	0.00	0.02	0.00	0.03
131	0.00	0.04	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00
135	0.00	0.04	0.04	0.06	0.00	0.00	0.03	0.00	0.00	0.00	0.04	0.00	0.00
139	0.00	0.00	0.00	0.06	0.03	0.00	0.00	0.00	0.02	0.03	0.06	0.00	0.03
143	0.00	0.04	0.00	0.00	0.00	0.04	0.10	0.00	0.00	0.03	0.00	0.00	0.03
147	0.00	0.00	0.04	0.03	0.13	0.04	0.08	0.00	0.00	0.08	0.02	0.06	0.03
151	0.00	0.00	0.02	0.09	0.08	0.04	0.10	0.03	0.17	0.11	0.00	0.15	0.03
155	0.00	0.04	0.04	0.06	0.03	0.00	0.03	0.03	0.00	0.00	0.06	0.00	0.00
159	0.00	0.00	0.04	0.00	0.00	0.00	0.05	0.05	0.04	0.00	0.00	0.00	0.00
163	0.00	0.00	0.00	0.00	0.03	0.00	0.10	0.00	0.00	0.11	0.00	0.06	0.03
167	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.03
Ho	0.88	1.00	1.00	0.94	0.84	1.00	1.00	0.90	0.88	0.95	0.92	0.82	0.89
He	0.72	0.87	0.89	0.89	0.89	0.87	0.88	0.84	0.87	0.89	0.89	0.89	0.89
Fis	-0.21	-0.15	-0.12	-0.05	0.05	-0.15	-0.13	-0.08	-0.01	-0.06	-0.03	0.07	0.01
N	16	13	23	17	19	12	20	20	24	19	25	17	18

OMM1082

Alleles (in bp)	FC	JC	MNF	LNF	UIC	MIC	LIC	UAC	MAC	LAC	URC	MRC	LRC
176	0.00	0.00	0.02	0.00	0.00	0.04	0.03	0.00	0.00	0.00	0.00	0.00	0.06
180	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00
184	0.03	0.00	0.02	0.03	0.00	0.08	0.00	0.03	0.08	0.05	0.06	0.06	0.06
188	0.00	0.04	0.04	0.15	0.16	0.08	0.08	0.10	0.15	0.00	0.16	0.15	0.08
192	0.00	0.00	0.02	0.09	0.03	0.12	0.00	0.05	0.04	0.26	0.00	0.24	0.22
196	0.03	0.04	0.11	0.06	0.18	0.08	0.05	0.15	0.17	0.13	0.18	0.15	0.19
200	0.31	0.15	0.07	0.00	0.13	0.00	0.35	0.13	0.06	0.03	0.04	0.06	0.11
204	0.22	0.08	0.15	0.12	0.37	0.19	0.30	0.23	0.23	0.24	0.14	0.29	0.11
208	0.03	0.04	0.07	0.00	0.00	0.31	0.03	0.00	0.04	0.03	0.06	0.00	0.08
212	0.00	0.19	0.02	0.03	0.00	0.00	0.03	0.00	0.00	0.03	0.06	0.00	0.00
216	0.09	0.23	0.30	0.12	0.03	0.08	0.00	0.00	0.08	0.11	0.06	0.06	0.03
220	0.28	0.04	0.04	0.21	0.00	0.04	0.13	0.15	0.04	0.00	0.02	0.00	0.00
224	0.00	0.00	0.00	0.06	0.03	0.00	0.03	0.00	0.06	0.03	0.10	0.00	0.00
232	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00
236	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00
238	0.00	0.19	0.11	0.09	0.03	0.00	0.00	0.00	0.02	0.00	0.02	0.00	0.03
240	0.00	0.00	0.00	0.03	0.05	0.00	0.00	0.05	0.00	0.05	0.08	0.00	0.03

OMM1082 (continued)

Alleles (in bp)	FC	JC	MN	LNF	UIC	MIC	LIC	UAC	MAC	LAC	URC	MRC	LRC

			F										
244	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00
Ho	0.94	0.85	0.91	0.94	0.74	0.69	0.90	0.85	0.83	0.90	1.00	0.88	0.94
He	0.76	0.84	0.85	0.88	0.78	0.83	0.76	0.86	0.87	0.84	0.89	0.80	0.87
	-	-	-										
Fis	0.23	0.01	0.08	-0.07	0.06	0.16	-0.18	0.01	0.04	-0.07	-0.13	-0.10	-0.09
N	16	13	23	17	19	13	20	20	24	19	25	17	18

OMM1087

Alleles (in bp)	FC	JC	MN F	LNF	UIC	MIC	LIC	UAC	MAC	LAC	URC	MRC	LRC
213	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00
241	0.22	0.12	0.00	0.09	0.03	0.04	0.18	0.08	0.04	0.08	0.20	0.25	0.03
245	0.03	0.15	0.08	0.09	0.21	0.27	0.30	0.11	0.10	0.13	0.16	0.19	0.06
249	0.00	0.00	0.02	0.00	0.03	0.00	0.05	0.00	0.04	0.00	0.02	0.00	0.00
253	0.38	0.15	0.19	0.12	0.05	0.00	0.00	0.03	0.00	0.00	0.02	0.00	0.03
257	0.34	0.31	0.21	0.32	0.16	0.27	0.05	0.05	0.23	0.11	0.08	0.22	0.31
261	0.00	0.04	0.04	0.00	0.03	0.04	0.15	0.32	0.10	0.05	0.10	0.03	0.11
265	0.03	0.00	0.02	0.09	0.11	0.12	0.05	0.05	0.02	0.00	0.10	0.06	0.17
269	0.00	0.00	0.23	0.18	0.18	0.08	0.08	0.13	0.13	0.18	0.04	0.11	0.00
273	0.00	0.08	0.02	0.06	0.08	0.04	0.05	0.11	0.13	0.11	0.08	0.03	0.17
277	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.04	0.08	0.04	0.00	0.06
281	0.00	0.08	0.02	0.03	0.05	0.12	0.05	0.03	0.10	0.08	0.04	0.08	0.03
285	0.00	0.08	0.06	0.03	0.00	0.04	0.03	0.05	0.02	0.13	0.12	0.03	0.03
289	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.03	0.02	0.03	0.00	0.00	0.00
291	0.00	0.00	0.08	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03
293	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.03	0.00	0.03	0.00	0.00	0.00
Ho	1.00	1.00	0.88	0.88	0.84	1.00	0.75	0.90	0.88	0.90	0.84	1.00	0.67
He	0.69	0.83	0.85	0.82	0.87	0.82	0.84	0.84	0.88	0.89	0.88	0.83	0.83
	-	-	-			-							
Fis	0.45	0.21	0.03	-0.07	0.03	0.23	0.10	-0.06	0.00	-0.01	0.05	-0.21	0.20
N	16	13	24	17	19	13	20	19	24	19	25	18	18

Table 1-8. Observed Heterozygosities (H_o) for 11microsatellite loci and 13 populations of Navarro River steelhead. H_o values that deviated significantly from Hardy-Weinberg expectations after correction for multiple tests (α=0.0003) are sown in boldface.

Loci	Populations													**Locus**
	FC	JC	MNF	LNF	UIC	MIC	LIC	UAC	MAC	LAC	URC	MRC	LRC	**Average**
OtsG3	0.31	0.62	0.54	0.35	0.47	0.23	0.39	0.35	0.62	0.53	0.52	0.19	0.44	0.44
OtsG43	0.93	0.77	0.90	0.94	0.61	0.36	0.90	0.80	0.75	0.95	0.72	0.67	0.72	0.77
OtsG83b	0.94	1.00	0.88	1.00	1.00	1.00	0.95	0.95	0.71	0.79	0.96	0.93	1.00	0.93
OtsG85	0.94	1.00	0.92	0.94	0.84	0.85	0.85	0.70	0.92	0.95	0.92	0.82	0.89	0.89
OtsG243	0.19	0.77	0.83	0.53	0.79	0.69	0.60	0.75	0.54	0.90	0.60	0.71	0.71	0.66
OtsG249b	1.00	1.00	0.79	0.88	0.72	0.75	0.85	0.80	0.96	0.84	0.84	0.82	1.00	0.87
OtsG253	**0.94**	0.92	0.87	0.81	0.79	0.77	0.85	0.95	1.00	0.90	0.91	0.79	0.93	0.88
OtsG401	0.62	0.85	0.88	0.82	0.90	0.85	0.90	0.79	0.79	0.95	0.88	0.83	0.78	0.83
OtsG423	0.88	1.00	1.00	0.94	0.84	1.00	1.00	0.90	0.88	0.95	0.92	0.82	0.89	0.92
OMM1082	0.94	0.85	0.91	0.94	0.74	0.69	0.90	0.85	0.83	0.90	1.00	0.88	0.94	0.88
OMM1087	1.00	1.00	0.88	0.88	0.84	1.00	0.75	0.90	0.88	0.90	0.84	1.00	0.67	0.89
Population Average	0.84	0.89	0.85	0.82	0.78	0.74	0.81	0.79	0.81	0.87	0.83	0.77	0.82	----

Table 1-9. Analysis of molecular variance (AMOVA) test results for combined data set of 11 loci and pooled steelhead samples from six Navarro River tributaries. All variance components were significant at the *P*=0.001 level.

Source of Variation	d.f.	Sum of Squares	Variance components	Percentage of Variation
Among Populations	5	72.92	0.13 Va	2.92
Within Populations	480	2110.93	4.40 Vb	97.08
Total	485	2183.86	4.53	----

Table 1-10. Pairwise population F_{st} values for the combined data set of 11 microsatellite loci and pooled steelhead samples from six Navarro River tributaries. All pairwise population F_{st} values were significant after correction for multiple tests (α=0.003).

	FC	JC	NF	IC	AC	RC
FC	----					
JC	0.10	----				
N	0.08	0.03	----			
IC	0.10	0.03	0.02	----		
AC	0.10	0.03	0.02	0.01	----	
RC	0.10	0.03	0.02	0.02	0.01	----

Table 1-11. Analysis of molecular variance (AMOVA) test results for combined data set of 11 loci and 13 populations of Navarro River steelhead. All variance components were significant at the *P*=0.001 level.

Source of Variation	d.f.	Sum of Squares	Variance components	Percentage of Variation
Among Groups	4	63.33	0.08 Va	1.89
Among Populations within Groups	8	62.26	0.09 Vb	2.03
Within Populations	473	2058.28	4.35 Vc	96.08
Total	485	2183.86	4.53	----

Table 1-12. Pairwise population F_{st} values for the combined data set of 11 microsatellite loci and 13 populations of Navarro River steelhead. Pairwise population F_{st} values significant at the after correction for multiple tests (α=0.0006) are given in boldface.

	FC	JC	MNF	LNF	UIC	MIC	LIC	UAC	MAC	LAC	URC	MRC	LRC
FC	**----**												
JC	**0.10**	**----**											
MNF	**0.09**	**0.04**	**----**										
LNF	**0.07**	**0.02**	**0.01**	**----**									
UIC	**0.11**	**0.03**	**0.02**	**0.02**	**----**								
MIC	**0.12**	**0.04**	**0.04**	**0.03**	**0.03**	**----**							
LIC	**0.12**	**0.05**	**0.04**	**0.04**	**0.03**	**0.05**	**----**						
UAC	**0.11**	**0.05**	**0.03**	**0.03**	0.01	**0.04**	**0.04**	**----**					
MAC	**0.11**	**0.03**	**0.01**	**0.02**	0.01	**0.03**	**0.03**	**0.01**	**----**				
LAC	**0.12**	**0.04**	**0.03**	**0.03**	0.01	**0.03**	**0.04**	**0.02**	**0.01**	**----**			
URC	**0.11**	**0.04**	**0.03**	**0.03**	**0.01**	**0.04**	**0.05**	**0.02**	**0.01**	**0.02**	**----**		
MRC	**0.11**	**0.03**	**0.04**	**0.02**	**0.02**	**0.04**	**0.04**	**0.03**	0.01	**0.02**	**0.02**	**----**	
LRC	**0.11**	**0.03**	**0.03**	**0.02**	0.02	**0.03**	**0.04**	0.02	0.01	0.01	0.01	0.01	**----**

Table 1-13. Assignment of steelhead individuals from six Navarro River tributaries based on 11 microsatellite loci. Boldface values on the diagonal represent the proportion of individuals correctly assigned to their source population.

	Assigned Population					
Source Population	FC	JC	NF	IC	AC	RC
FC	**0.94**	---	0.06	---	---	---
JC	---	**1.00**	---	---	---	---
NF	0.10	0.26	**0.39**	0.10	0.10	0.05
IC	0.04	0.19	0.06	**0.52**	0.13	0.06
AC	0.03	0.27	0.08	0.13	**0.32**	0.17
RC	0.08	0.28	0.03	0.03	0.17	**0.43**

Table 1-14. Assignment tests of ten steelhead collected from the Navarro River estuary and assigned to one of 6 Navarro River Tributaries. LOD scores > 2.0 have a 1/100 chance of error or less (P<0.01). FC=Flynn Creek, JC=John Smith Creek, NF=North Fork, IC=Indian Creek, AC=Anderson Creek, and RC=Rancheria Creek.

	Assigned Population		
Sample	Most Likey # 1	Most Likely # 2	LOD Score
NE1	FC	IC	18.06
NE2	JC	IC	22.45
NE3	FC	RC	1.22
NE4	FC	RC	9.76
NE5	JC	RC	24.92
NE6	NF	AC	10.93
NE7	RC	AC	156.30
NE8	NF	RC	1.72
NE9	NF	JC	2240.00
NE10	RC	JC	1.76

Figure 1-16. Unweighted pair group method with arithmetic means (UPGMA) dendrogram of Nei's (1972) genetic distances based on pooled steelhead samples for six Navarro River tributaries. Bootstrap values at the nodes indicate the percentage of times populations beyond the node grouped together based on 1,000 bootstrap iterations.

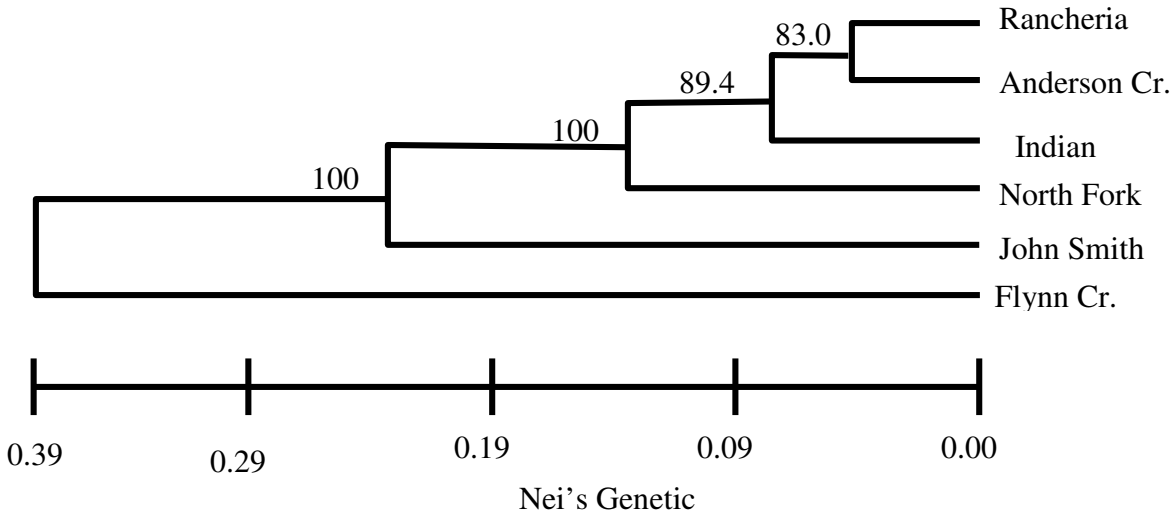
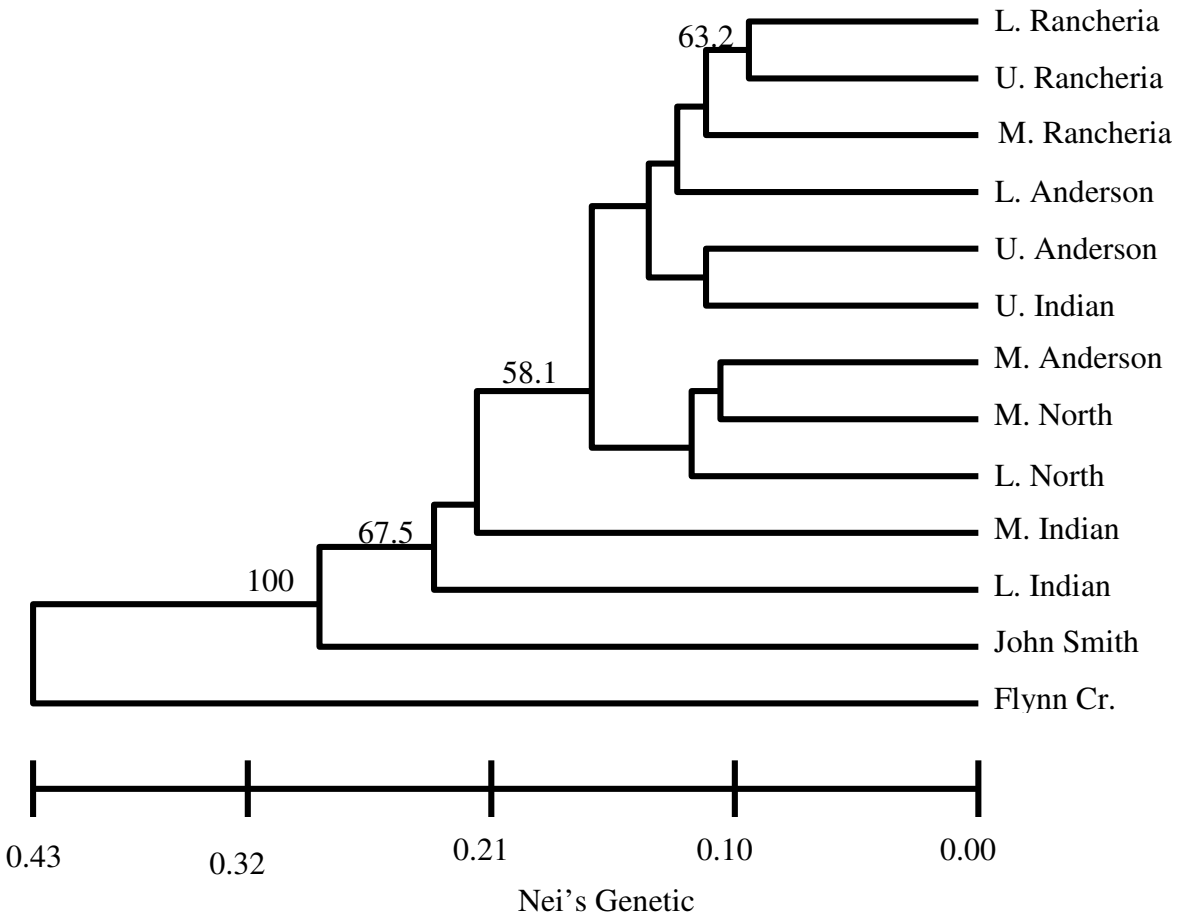


Figure 1-17. Unweighted pair group method with arithmetic means (UPGMA) dendrogram of Nei's (1972) genetic distances for 13 discrete steelhead samples of taken in six Navarro River tributaries. Bootstrap values at the nodes indicate the percentage of times populations beyond the node grouped together based on 1,000 bootstrap iterations.



Fish Community Structure

Understanding the structure and function of fish communities in which salmonids reside in the watershed, is an essential step before the effects of any stressor can be evaluated. Our primary goal was to focus on the trophic structure and transfer of energy to the salmonids who serve as the top predators. The importance of the transfer of energy is clear, in that the feeding and growth rates of juvenile salmonids determines the size at which the fish smolt. Size at smoltification and size at outmigration to the open ocean is a primary determinant of the survival of the fish during the early pelagic phase of its life.

Introduction

Community studies often grapple with complexities introduced by arbitrary community boundaries, predictions derived from unnaturally discrete trophic levels, and problems tracing detrital energy flows. These features complicate identification of processes that determine species distribution and the effects of disturbance. The link between observed patterns and processes in dynamic systems requires data that delineate relevant spatial scales of interaction, accurately measure community traits such as food chain length, and assess the importance of detrital energy sources. These data are especially important in interconnected and dynamic systems such as streams.

Defining Community Boundaries

A persistent problem in community ecology has been the identification of relevant community boundaries. Some community definitions use geographic or abiotic features of a habitat to identify community boundaries (Emlen 1977, Ricklefs 1990). This approach provides clear physical boundaries, but organisms and energy often have no

fidelity to such partitions. Neglect of energy and organisms that move between habitats limits understandings of local process and ecological prediction (Polis and Hurd 1997). Interactions among groups of organisms may also define community boundaries (Whittaker 1977, Price 1984). This definition provides an ecologically relevant means to test community membership, but also faces difficulties. In experiments and empirical studies, logistical constraints typically limit the operational definition of "interaction communities" to a small sub-set of organisms from a larger group of interacting species (Brown 1994, Ulanowicz 1997). Both the interaction and geographic community definitions risk excluding organisms vital to ecological processes.

One solution proposed for the delineation of ecosystem boundaries has been the use of top predators as a spatial and temporal benchmark (reviewed in Cousins 1997). In this approach, top predators are considered an "energetic sink" whose spatial range defines ecosystem boundaries. The "sink-food web" or Ecosystem Trophic Module (ETM) community approach provides an objective, quantifiable spatial setting for local observations, and linking community and ecosystem processes. This approach may be especially useful in dynamic systems such as streams that lack distinct longitudinal boundaries.

Stable isotope analysis (SIA) provides a tool to identify energy sources and delineate community boundaries. Heavy carbon (^{13}C) enters aquatic food webs at different rates depending on conditions at the boundary layer where primary producers absorb inorganic carbon (Rounick and Winterbourn 1982). For instance, $\delta^{13}C$ measured in allochthonous

detritus and autochthonous stream algae often differ in predictable ways (Bunn et al. 1989, Junger and Planas 1994). Measurements of $\delta^{13}C$ in consumers reflect the $\delta^{13}C$ of their basal carbon sources, and thus provide a natural tracer of basal carbons sources. Isotopic signatures are conserved at rates determined by tissue turnover in organisms (Fry and Arnold 1982, L.L. Tieszen et al. 1983). Consequently, local differences in isotopic ratios can be used to infer spatial independence within populations and communities (France 1995, Taki and Sakamoto 1999). Differences in $\delta^{13}C$ among consumers, whether derived from different source carbons or not, reflect differences in basal carbon sources, and thus a type of community boundary.

Making Trophic Levels Realistic

Food chain length constitutes one of the few general, emergent ecological properties widely acknowledged to affect community and ecosystem function (Hairston et al. 1960, Fretwell 1977, Hairston and Hairston 1996, Bengtsson and Martinez 1997). Many community models make predictions based on numbers of trophic transfers that occur between a basal energy source and a top predator. However, groups of organisms that feed exclusively at discrete trophic levels are heuristic constructs at best (Cousins 1987, Hairston and Hairston 1997). Realistic descriptions of food chain length require more precise quantitative tools.

Trophic spectra have been proposed as an alternative to discrete trophic levels (Oksanen et al. 1981, Strong 1992, Strong and Polis 1996). Trophic spectra assign a numerical position to organisms in the food chain based on the average number of trophic transfers

between them and the source carbon of their diet. Ratios of ^{14}N to ^{15}N in the tissues of animals in the food web provide continuously distributed data for food chain position amenable to analysis of the trophic spectrum. Studies across lake ecosystems show that the concentration of ^{15}N increases by approximately 3.4 $^{0}/_{00}$ at each step in the food chain (Vander Zanden et al. 1997). Thus, concentrations of this stable isotope in organisms can provide an indirect measure of the trophic position. When measured in top predators, ^{15}N establishes total food chain length. Only a few analyses have used this technique (Kling et al. 1992, Vander Zanden et al.1999, Post et al. 2000).

Problems of community ecology in coastal Californian stream ecosystems

Community boundaries, energy sources and food chain length vary over time and space in lotic communities (Vannote et al. 1980, Power et al. 1990, Power et al.1997). Within North Coastal California streams, juvenile anadromous salmonids may act as a top predator that regulate community structure through trophic cascades during summer low flow conditions (Power et al. 1990). However, effects of fish size or allochthonous inputs on food chain length have not been explicitly addressed in these systems. Although smaller fish have been found in the stomachs of larger steelhead parr, it is not clear if larger fish constitute an additional trophic level. Predicted outcomes of cascading interactions will be altered if large juvenile salmonids or microbial processes form additional trophic step. This paper uses SIA to: 1) describe spatial differences in isotopes of top predators and invertebrates at the scale of the sub-watershed and stream reach, 2) compare consumer stable carbon signatures to environmental variables across a gradient

of stream sizes, and 3) report environmental correlates with late summer food chain length.

Methods

Ecological Data

We measured volumetric flow using the velocity-area method outlined by Gore (1996). Allochthony scores were created for each site through qualitative estimates of vegetative cover in consecutive pools and riffles ($6 \leq n \leq 10$). Each pool and riffle was assigned an allochthonous vegetation score from 0 to 5, with 0 = 0% cover to 5 = 100% cover. Allochthonous vegetation included all terrestrial-based organic matter that had settled onto the substrate within the wetted width of a pool or riffle. We measured velocity within each cross-section using a Marsh-McBirney Flo-Mate 2000 flow meter positioned at 0.6 times the water depth, measured from the water surface. Mean wetted width and water depth were measured using eleven stream cross sections uniformly distributed along the stream reaches. Water depth was measured three times at equal intervals at each cross section (mean width N = 11; mean depth N = 33). Site volume was determined as the product of the average riffle-pool sequence mean depth and width (N = 5 riffle pool sequences in 2^{nd} and 3^{rd} order streams, N = 3 in 4^{th} order streams).

Organism Sampling

We collected steelhead on August 4 and 5 1999 at all sites except the North Fork. Samples from the North Fork were collected on October 21, 1999. Fish were collected with a Smith-Root Model 12-B electrofisher at all sites except the North Fork where fish were collected with a 9.1 m beach seine. Steelhead were listed as a candidate for

threatened status in California, so collection of substantial numbers of large juveniles was not a possibility. However, during December of 1999 and January of 2000, we were able to collect four smolts (pre-migratory juveniles) from the Navarro River estuary using a 91 m beach seine, and three smolts by electrofishing from a school of smolts in a pool in the North Fork of the Navarro River. Contents of all fish stomachs were removed by dissection and contents were identified with enough taxonomic resolution to determine if contents were fish, or predatory invertebrates were present. Fish age was determined by examining sagittal otoliths.

We collected aquatic invertebrates using a Serber sampler or by removing individual invertebrates from rocks and debris by hand at each site except the North Fork on August 4 and 5. Isotopic analysis of individual hydropsychid Trichoptera was performed for each site. Additional invertebrates (gastropods of the family Ancylidae and Physidae and Coleoptera of the family Psephenidae) were collected by hand from pools on October 21, 1999 at all sites but Dimmick Park. The trichopteran *Gumaga sp*. was collected by hand from three riffles and three pools on November 14th at each of the seven sites. *Gumaga* sp. collected from a single riffle or pool was grouped for analysis.

Stable Isotope Analysis

Muscle tissue from individual fish and all invertebrates soft tissue (shells were excluded in the analysis of Mollusks) were oven-dried at approximately 60^{o}C for at least 72 hours and lightly ground to a powder. Isotopic analyses were performed at the Stable Isotope Facility at the University of California at Davis using a Europa Scientific Hydra 20/20 IRMS with an analytic precision of +/- 0.1 per mille for carbon and +/- 0.2 per mille for

nitrogen. Standards used for ^{15}N and ^{13}C analysis were calculated by standard methods using air, and Pee Dee Belemnite as standards respectively. $\delta^{15}N$ and $\delta^{13}C$ were calculated according to the equation $[((R_{sample}/R_{standard}) - 1) \times 1000]$ where R is $^{15}N/^{14}N$ or $^{13}C/^{12}C$. Tissue samples containing less than 10 μg of C or N demonstrated sample weight bias and were excluded from analysis. All statistical analyses were performed in SPSS 7.0. Food chain length was calculated as the difference between primary consumer and parr $\delta^{15}N$ values (Vander Zander et al. 1997).

Results

Detrital Isotopic Values

Detrital material including processed and unprocessed leaf material and wood was collected from benthic samples at each site on August 4th and 5th. The average $\delta^{13}C$ value was –28.86. Values ranged from -26.2 at Hendy Woods to –29.3 at Rancheria Creek (S.E. = 0.32, N=6 sites; detritus was not collected at the North Fork). Average $\delta^{15}N$ for detritus was -0.7 (S.E. = 0.52).

Invertebrate Stable Isotope Values

One-way analysis of variance (ANOVA) with Tukey post-hoc comparisons were used to evaluate differences in primary consumer isotopic concentrations between sites, taxa and collection dates. Invertebrate isotopic values varied across sites in the watershed ($\delta^{13}C$: One-way ANOVA; $F = 10.892$, $P < 0.000$; $\delta^{15}N$: One-way ANOVA; $F = 3.302$, $P = 0.026$). $\delta^{13}C$ was significantly higher at Hendy Woods on the Navarro River and the North Fork of the Navarro (both fourth order streams), and depleted in Flynn Creek (a second order stream). $\delta^{15}N$ values were higher in Flynn Creek and lower in the North

Fork of the Navarro. Isotopic concentrations did not differ between taxa (one-way ANOVAs; $F < 2.07$, $P > 0.128$), or collection date (one-way ANOVAs; $F < 0.714$, $P > 0.502$).

Steelhead Stable Isotope Values

One-way analysis of variance (ANOVA) with Tukey post-hoc comparisons were used to evaluate differences in steelhead parr isotopic concentrations between sites. Steelhead isotopic values varied across sites in the Navarro watershed ($\delta^{13}C$: One-way ANOVA; $F = 11.152$, $P < 0.000$; $\delta^{15}N$: One-way ANOVA; $F = 21.815$, $P < 0.000$). $\delta^{15}N$ was significantly lower at Hendy Woods on the Navarro River and at Dimmick Park on the Navarro (both fourth order streams), and higher in Flynn Creek (a second order stream). $\delta^{13}C$ values were lower in Flynn Creek and higher at Hendy Woods. Average size of 0-age steelhead was 75.3 mm (S.E. = 2.57) and 132.17 (S.E. = 6.08) for 1+ fish. $\delta^{13}C$ values were –24.92 (S.E. = 0.37) and –25.18 (S.E. = 0.31) for 0-age and 1+ fish respectively. $\delta^{15}N$ was 7.08 (S.E. = 0.22) and 7.93 (S.E. = 0.21) for 0-age and1+ fish respectively.

Average total length of smolts was almost twice the average size of parr in the Navarro River (North Fork, N= 3, 226.33mm total length, S.E. = 6.88: Estuary, N =3, 211.75 mm total length; S.E. = 7.28). Smolts at both sites had higher $\delta^{15}N$ values and enriched $\delta^{13}C$ relative to parr. Nonparametric analyses showed that both $\delta^{13}C$ and $\delta^{15}N$ were higher in the estuary than in the North Fork of the Navarro River (Mann-Whitney U; $Z = -2.212$; $P = 0.034$). SIA from the invertebrate scavenger Gammarus sp. collected from smolt

stomachs showed that higher $\delta^{13}C$ (-18.45, S.E. = 0.065, N = 5), and $\delta^{15}N$ (8.32, S.E = 0.34, N = 5), than typically occurred elsewhere in the watershed.

Steelhead diets

Juvenile steelhead contained a variety of diet items. Invertebrates categorized as predators included Hempiterans (family Corixidae, and Nacouridae), Odonates (family Gomphidae), Megalopterans (genus Sialis), Diptera (Tipulidae, genus Hexatoma), and Plecoptera. Invertebrate grazers included aquatic Lepidoptera (genus Petrophila), terrestrial and aquatic adult Coleoptera, Trichoptera, Ephemeroptera, Diptera (Chironomidae), Arachnids (Hydracarina), Amphipods, and Decapods. Fish occurred in the stomachs of 6 of 53 fish. The average total length of juvenile steelhead containing fish was 134.67mm (S.E. = 31.87), and without fish was 93.29mm (S.E. = 5.54). A logistic regression was used to determine the effect of steelhead total length on the probability that fish would occur in the diet. Overall model results were marginally significant (d.f. = 1, P = 0.050, $R^2 = 0.221$, $\beta = 0.0158$). Thus the likelihood juvenile steelhead will consume fish increased 1.58 % for each millimeter increase in total length. A logistic model examining the effect of steelhead size on the likelihood of predatory invertebrates occurring in the diet was not significant (d.f. = 1, P = 0.968, $R^2 = 0.000$).

Correlations Between Variables

A variety of relationships were apparent within $\delta^{15}N$ and $\delta^{13}C$ values for primary consumers and steelhead parr. Using station averages as replicates, invertebrate $\delta^{13}C$ and $\delta^{15}N$ were negatively related (d.f. = 5; R = -0.800, P = 0.031), as were steelhead $\delta^{13}C$ and $\delta^{15}N$ (d.f. = 5; R = -0.874, P = 0.010). $\delta^{13}C$ of invertebrates and steelhead collected

across the Navarro watershed and estuary were positively correlated (d.f. = 6, R = 0.834, P = 0.047). No trend was observed between average discharge (Q) and parr $\delta^{13}C$ (P = 0.399) or average velocity (Q/average habitat volume; P = 0.596). No trend was observed between steelhead parr size and $\delta^{13}C$ and $\delta^{15}N$ values when fish were considered individually (d. f. = 47, P > 0.214); however station averages of steelhead $\delta^{13}C$ were positively related to parr size (d.f. = 5, R = -0.887, P = 0.008).

Numerous correlations were observed between and among biotic and abiotic variables measured in this analysis. Log_{10} total drainage area was negatively related to allochthony ratings and steelhead parr $\delta^{15}N$. Stream reach volume was also positively related to steelhead $\delta^{13}C$ and negatively related to steelhead parr $\delta^{15}N$, and negatively related to invertebrate $\delta^{15}N$. Combined riffle and pool allochthony ratings averaged for each site over the summer were negatively related to steelhead $\delta^{13}C$ (d. f. = 5, R = -0.890, P = 0.007) and positively related to steelhead $\delta^{15}N$ (d. f. = 5, R = 0.867, P = 0.012).

Primary consumer $\delta^{15}N$ was subtracted from steelhead $\delta^{15}N$ to obtain a measure of relative food chain length in a modification of the methods used by Vander Zanden et al. (1998). No relationship was observed between average parr size and food chain length or drainage area (d. f. = 5, P > 0.223). Food chain length was positively related to allochthony scores (d. f. = 5, R = 0.753, P = 0.51). Food chain length also differed between 0 age and 1+ steelhead juveniles (t-test, P = 0.045).

Discussion

Anthropocentric definitions of communities set along habitat and taxonomic boundaries may neglect complex ecological processes and thus fail to provide a context for reliable predictions. Spatially unique isotopic signatures, and correlation between invertebrate and steelhead δ-^{13}C values indicate that in Navarro Watershed and estuary, local environmental signals systematically determine consumer isotopic signatures. Steelhead δ-^{13}C values are related to the volume of habitats and the drainage area of the watershed they occupy. Boundaries of top predator ranges, and therefore the boundaries of sink-web communities (*sensu* Cousins 1997) appear to be limited during late summer. Movement of invertebrates and parr may be limited at this time by low flow and shallow riffles in streams. Sink-web models of community boundaries may have some utility in the Navarro watershed for delineating coarse spatial independence between sub-watersheds over defined periods of time.

Possible ecological sources of isotopic patterns in steelhead and invertebrates

According to the stream continuum concept, the importance of microbial heterotrophic and photosynthetic autotrophic processes should differ with stream order (Vannote et al. 1980). In headwater streams allochthonous inputs of leaf and other plant material is processed by heterotrophic fungi and bacteria (reviewed in Hauer and Lamberti 1996). Autochthonous production of periphyton becomes more important as stream size increases, stream banks widen and more light energy reaches the stream substrate (Vannote et al. 1980). Patterns in the Navarro suggest microbial processes are more important in smaller streams.

Among steelhead and invertebrates in the Navarro watershed, ^{15}N was more depleted in the presence of enriched $\delta^{13}C$ signatures. Heavy nitrogen becomes enriched during microbial denitrification (Cline and Kaplan 1975). Anaerobic microbes also deplete ^{13}C (Rich and Wetzel 1978, Rau 1979). We hypothesize that microbial processes may be an important source of the patterns observed here. Similar mechanisms have been suggested in lake environments that show similar patterns of depleted N and enriched C occurring in hypolimnetic zones (Vander Zanden and Rasmussen 1999). The fact that steelhead and detrital isotopic signatures were equal in smaller streams, and $\delta^{13}C$ in invertebrates and steelhead systematically increases along a gradient of stream size points to the potential importance of microbial processes in smaller streams. However, all potential sources of production were not identified, so this conclusion must remain tentative.

Invertebrate and steelhead $\delta^{13}C$ values differed in Flynn Creek, indicating that the carbon sources utilized by aquatic invertebrates were different from those used by steelhead in this system. Diet analysis at this site showed that terrestrial diet items were uncommon at most sites, but 57% of steelhead collected at Flynn Creek contained terrestrial insects. These insects would have contained a terrestrial carbon signature similar to the one observed in detritus from this site. Thus, steelhead consuming terrestrial insects may display a $\delta^{13}C$ value similar to that of terrestrial producers. Additional analysis will test this hypothesis. Please note the removal of the Flynn Creek data from this study does not alter the trends reported from the watershed.

We see no evidence of systematic bias from productivity, marine allochthony, velocity, temporal changes or temperature that may confound our correlative analysis. Carbon-13 becomes enriched in autotrophs as competition for inorganic carbon increases during periods of high productivity and our larger, more productive sites contained enriched $\delta^{13}C$ values (Schindler et al. 1998, MacLeod and Barton 1998, Findlay et al. 1999). However, ^{15}N also becomes enriched in productive systems (MacLeod and Barton 1998) and our data showed the opposite trend across productivity gradients. Marine carbon from spawning anadromous fish may also enrich $\delta^{13}C$ signatures, but again this normally occurs in conjunction with enriched ^{15}N signatures. In analyses from a nearby watershed, $\delta^{13}C$ values of invertebrates from pools were higher than riffle values among invertebrates (Finlay et al. 1999). In our study, neither invertebrate or steelhead δC_{13} was related to discharge or average velocity at each site. Isotopic signatures of the trichopteran Gumaga sp. between pools and riffles in the Navarro River in October showed no differences within or across sites (H. Sarakinos and T. Smith, unpublished data). Invertebrates were collected over a period of three months, but did not show temporal trends in either ^{13}C or ^{15}N. Temperatures were also lower and shaded cover was higher in low order streams (J. Feliciano, unpublished data). Lower temperatures reduced rather than increase $\delta^{15}N$ values. With the exception of microbial processes, these error sources would counteract the trends in $\delta^{15}N$ and $\delta^{13}C$ observed in this analysis and thus are not likely to account for the trends observed.

Effects of fish size on food chain length: where is the top?

Food chain length varied with fish size in the Navarro Watershed. Fish sizes influence diet (reviewed in Gerking 1994), and may directly affect the function of the Navarro River ecosystem during the late summer. Previous studies of food chain length in North Coastal streams have described juvenile steelhead as a single trophic level (Power et al. 1997). Food chain length did not differ with average parr size, but food chain lengths to 1+ fish were an average of 1.04 delta units (or 0.3 trophic levels) higher than chain lengths to 0+ age fish. In the North Fork, average $\delta^{15}N$ values of smolts differed from parr by a full trophic level (3.8 $\delta^{15}N$ units). Thus, larger fish appear to consume organisms from higher trophic levels. Results of logistic regression from diet analysis suggest fish may be an important source of higher trophic status among large parr.

Intraguild predation by large juvenile steelhead extends food chain length in North Coast California streams and alters predictions of linear trophic models (Power et al. 1997). If cascading interactions occur in these systems, abundant large juvenile steelhead should reduce periphytic algae by consuming smaller fish that eat herbivorous invertebrates. However, omnivory occurs within the fish assemblage, and both small fish and invertebrates are consumed (Moyle 2000). The effects of fractional increases in food chain length have not been studied empirically and remain an open question. Additional analyses will be needed to test these interactions.

Several confounding influences must be considered in our analysis. Effects of smoltification on isotopic ratios are not known (although we see no reason this process

should systematically bias isotopic ratios). Smolts were also collected up to four months later than invertebrates, and seasonal changes in stable isotopes are possible (MacLeod and Barton 1998). However, in this case, changes over time should be minimal given the large size of fish and the resulting low tissue turnover. Higher smolt isotopic ratios also oppose expected trends of lower $\delta^{15}N$ levels during winter (Mcleod and Barton 1998).

Linking empirical trends to food chain theory

Theory and empirical observations suggest that ecosystem size and volume may act as determinants food chain length (Briand and Cohen 1987, Schoener 1989, Post et al. 2000). Interpretations of observed food chain length in streams depend explicitly on the defined boundaries around communities and ecosystems. In the case of Navarro Watershed data, food chain length varied across sites and mean values for juvenile steelhead were not related to habitat volume or drainage area. Unfortunately, some ambiguity remains in the interpretation of these trends.

Chain length increases at the top of the food chain with juvenile steelhead size, and increases at the bottom of the food chain with the abundance of allochthonous material (3.2 delta units difference). Mechanisms explaining increasing food chain length in the presence of greater allochthony are not immediately obvious. However, if causation exists for this pattern, longer food chains would be expected in smaller streams that receive greater amounts of allochthony. Trends observed to date do not show net changes in food chain length are clearly linked to habitat volume. Further work using

stable isotopes will need to account for the role of fish size and microbial activity on food chain length, and extend these findings over larger spatial and temporal scales.